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THE INTERACTION BETWEEN BREEDING AND GROWTH RATE IN THE BARNACLE *ELMINIUS MODESTUS* DARWIN

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ABSTRACT

Elminius modestus Darwin is shown to be a slightly protandrous hermaphrodite in which the testes, vesiculae seminales and penes develop in specimens measuring between 3 and 5 mm, and the ovaries in specimens measuring from 4 to 6 mm. Fast growing specimens mature and bear embryos within 6 to 7 weeks of settlement, but crowding may slow down growth and delay the maturation of the ovary considerably. Very slow growing specimens may eventually become mature at an abnormally small size.

When individuals were prevented from breeding by isolation but were grown otherwise under identical conditions, they grew significantly faster and to a larger size than individuals which were able to cross-fertilize and breed. The valves of the shell showed less difference in growth rate than any other structure. The slower growth of breeding individuals was caused not by competition due to their closer proximity, but by loss of tissue as egg masses. The mean difference in tissue weight, excluding shell, was about equal to the calculated loss of tissue in the form of eggs. Details are given of wet and dry weights and of the weights of the individual shell compartments of this species.

INTRODUCTION

The growth rate of barnacles is influenced by a number of factors, such as tidal level (Barnes and Powell 1953), water flow, orientation to current, population density, and parasitization (Crisp 1960). No experiments have yet been made to determine whether breeding has any effect on growth.

In many animals, particularly in fish and lamellibranchs, breeding is often accompanied by a reduction in growth or in tissue weight relative to length. Such halts in growth may be permanently recorded as bands of differential deposition in such structures as the otoliths and the shells.

Barnacles offer a unique opportunity for a study of the effect of breeding on growth under natural conditions, since it is possible to prevent breeding without altering other factors. A northern species such as *Balanus balanoides* (L.) is not suitable for such a study, since it breeds once only each year. The investigation was therefore carried out on *Elminius modestus* Darwin, a fast growing species which matures within a few weeks of settlement (Crisp and Chipperfield 1948) and breeds continuously (Crisp and Davies 1955). This species is an obligatory cross-fertilizing hermaphrodite, and breeding can be prevented by isolating individuals by a distance of 5 cm or more (Crisp 1958).

METHODS

Three pairs of individuals 2 cm apart and 6 isolated individuals separated by at least 5 cm were allowed to settle on panels of non-toxic plastic (Tufnol) each measuring 15×20 cm (Fig. 1A). The pattern of settlement was predetermined by drilling small pits, into which the cyprids settle preferentially, as described previously for *B. balanoides* (Crisp 1960). The pairs and single-

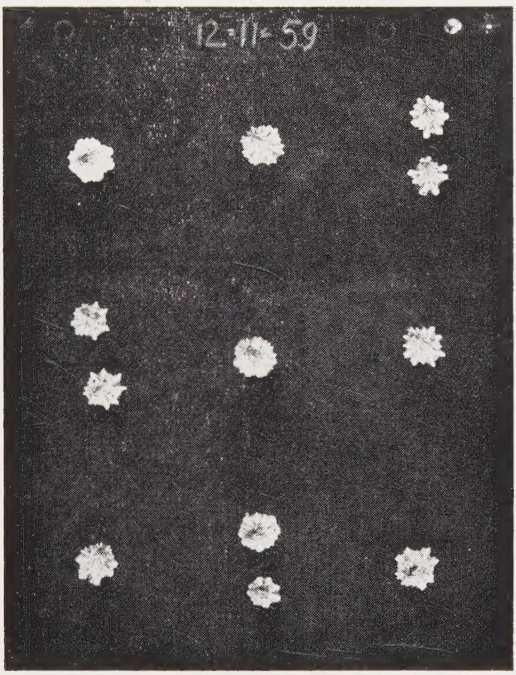
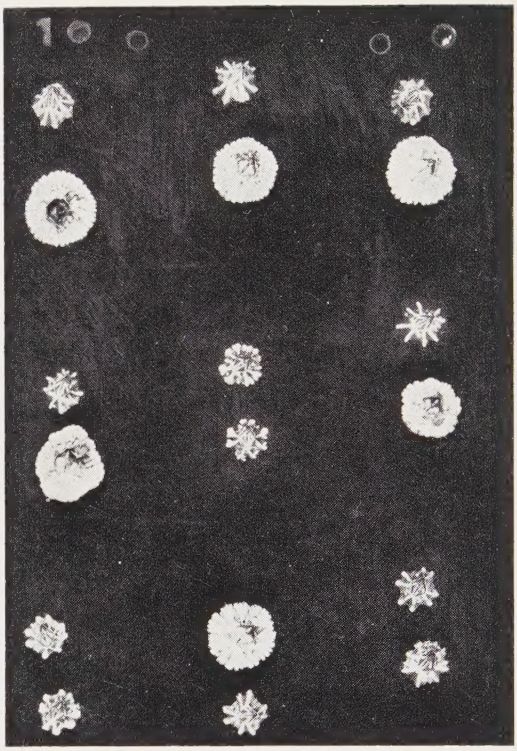


FIG. 1A. Pairs and singletons of *Elminius modestus* which settled in 1959 in a predetermined pattern on a Tufnol panel, and were kept cleared of other fouling growth for the duration of the experiment. This panel corresponds to No. 4 of the complete experimental set of nine panels.



B. Similar experiment in 1960 with isolated *Elminius* paired with *B. balanoides.*

tons were distributed among each of the 9 possible positions on the panels in a completely balanced design, as shown in Figure 2. The whole set of 9 panels was suspended from a raft moored in the Menai Straits and examined at intervals of two or three weeks during the summer and autumn of 1959. At each examination all additional fouling growth was removed and a photograph taken from which measurements were made subsequently; this operation took only a few hours and the barnacles could be returned to the sea on the same day.

After the final examination all the barnacles were removed, examined for the presence of egg masses, and weighed after blotting off superfluous water. Egg masses were removed from those containing them and weighed separately. The barnacles were then dried for 2 hr at 110°C and weighed again. Finally they were digested in hot potash until only the shell plates remained and these were washed in distilled water, dried and weighed separately. All weighings were carried out on a torsion balance using an optical lever to increase its sensitivity.

The breeding of *E. modestus* was studied by methods similar to those previously described in the literature. Gonad condition was assessed visually by examining specimens recently preserved in 10% formalin-sea water under a low power stereoscopic microscope using criteria previously given for *Balanus porcatus* (Crisp 1954). The penis was measured in the normal state of contraction found in preserved material by gently stretching it between two needles over a millimetre scale. Ova were placed in the minimum depth of water on a microscope slide, and measured under a low power objective with a high magnification callibrated Kellner eyepiece.

The age at which maturity was reached was determined by allowing *Elminius* to settle on inert panels in various habitats

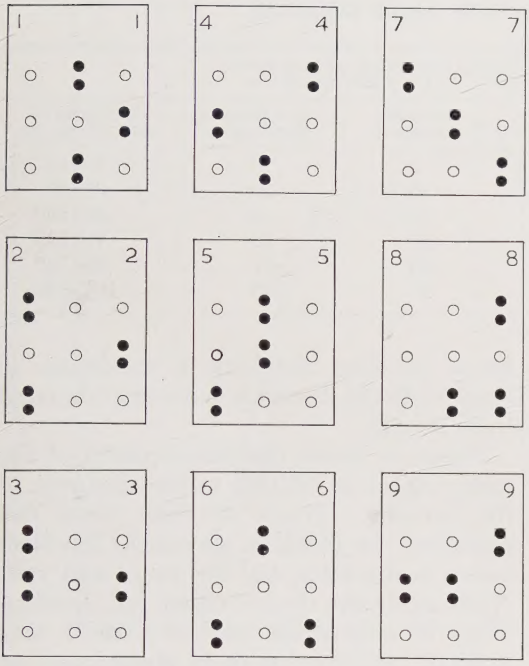


FIG. 2. The complete experimental design of the 1959 experiment in which pairs and singletons were equally represented on all nine positions of the panel. ●—Breeding individuals in pairs 2 cm apart. ○—Non-breeding individuals, as singletons, set at least 5 cm from any other individual.

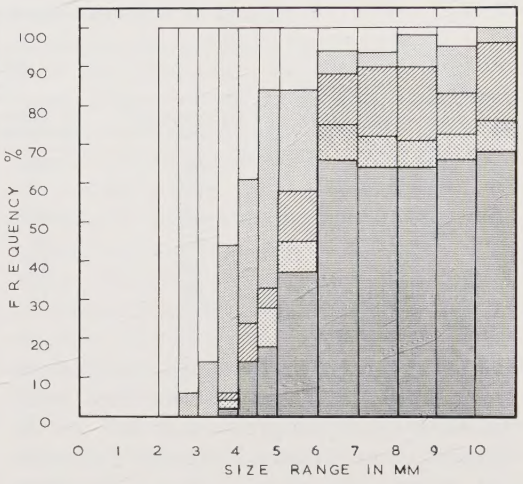


FIG. 3. Development of the gonads in a typical intertidal population of *Elminius modestus*.

Development of ovary, showing the frequency of individuals of each size group in which:

- No ovarian tissue was visible
- Ovary was thin, milky appearance and poorly developed
- Ovary was yellow, moderately well developed
- Ovary was large, filling much of mantle space
- Fertilized egg masses were present

and examining some hundred specimens at intervals. Some of the panels used were of glass to allow continuous inspection of gonad condition (Crisp and Davies 1955).

OBSERVATIONS ON THE BREEDING OF *ELMINIUS MODESTUS*

A detailed analysis of the frequency of broods throughout the year has already been made (Crisp and Davies 1955). It was found that a high proportion of specimens exceeding 6 mm in diameter bore fertilized eggs throughout the summer, but of those of less than 5 mm diameter, the proportion which bore eggs fell sharply with decreasing size. Figure 3 is based on a number of examinations of groups of 30 to 40 individuals taken during the summer months from a number of localities in southern England and Wales. The specimens from all localities appeared to breed similarly in relation to their size and the results were therefore pooled to represent the behavior of a naturally occurring population containing a proportion of both freely growing and crowded individuals.

In Figure 3 the degree of ovarian development is shown as a function of the rostrocarinal diameter. Only a very small proportion of specimens of about 3 mm diameter showed any trace of ovarian development, and fertilized eggs were rarely present in specimens of less than 4 mm. Between 4 and 6 mm the proportion bearing eggs increased abruptly with increase in size, and on reaching 6 mm the great majority, if not all, had become fully mature. It can be seen that, of the mature population, about two thirds carry embryos, and the remaining third have an ovary in varying degrees of regeneration. No account has been taken in presenting these data of the presence of

TABLE 1. *Ovarian regeneration in mature individuals*

Stage of development of embryos in mantle space (Crisp 1954)	Percentage of individuals with egg masses as in column 1 in which the ovary is				
	(a) Apparently absent	(b) Poorly developed	(c) Half developed	(d) Filling the mantle space	Size range of eggs in ovary (μ)
1–4	65	26	9	0	20–40
5–7	78	7	15	0	30–60
8–10	48	29	15	8	40–100
11–12	32	21	28	19	70–120
13	29	18	26	27	80–120
Nauplii liberated	20	17	27	36	100–150

ovaries in gravid individuals, but if these are included it is at once clear that, while the embryos of the previous brood are developing, a new ovary is steadily enlarging and its ova maturing. Table 1 illustrates this by classifying gravid individuals as a function of the stage of development of the previous brood. The percentage frequency of four stages in the degree of ovarian regeneration is compared for each group; the ovary shows a steady increase as the embryos develop, reaching a maximum in those individuals which have just liberated their nauplii.

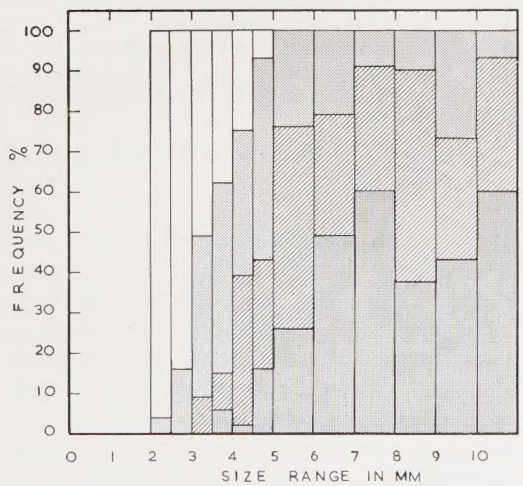


FIG. 4. Development of the testis and vesiculae seminales showing the frequency of individuals of each size group in which:

No trace of testis or vesiculae seminales were visible

Traces of testis tissue & vesiculae seminales were present

Testes & vesiculae seminales were moderately developed

Testes were well developed & v.s. swollen and turgid

Figure 4 shows the development of the male organs in relation to the diameter of the barnacle. Traces of testis tissue can sometimes be found in specimens less than 3 mm in diameter, but the testes and vesiculae seminales do not reach full development in individuals of less than 5 mm. Animals of 5 mm or more always contain male organs throughout the summer.

Figure 5 illustrates the development of the penis. This organ is absent in very small specimens and develops heterogonically in specimens of 3 to 5 mm diameter while the male organs are forming, thereafter growing more slowly. *Elminius* is therefore slightly protandrous. The male organs appear in the majority of specimens at a diameter of 3 to 4 mm and reach maturity at about 5 mm, while the female organs appear at 4 to 5 mm and reach maturity in specimens of about 6 mm.

THE INFLUENCE OF GROWTH CONDITIONS ON BREEDING

Crisp and Davies (1955) studied the percentage of ovigerous individuals in young growing populations situated at the center and at the edge of a group; they found that both the growth rate and the proportion carrying eggs were greater at the edge, where presumably there was less competition for food, than at the center.

The question therefore arises whether, under adverse conditions of growth, there is any significant change in the relationship between size and the onset of maturity.

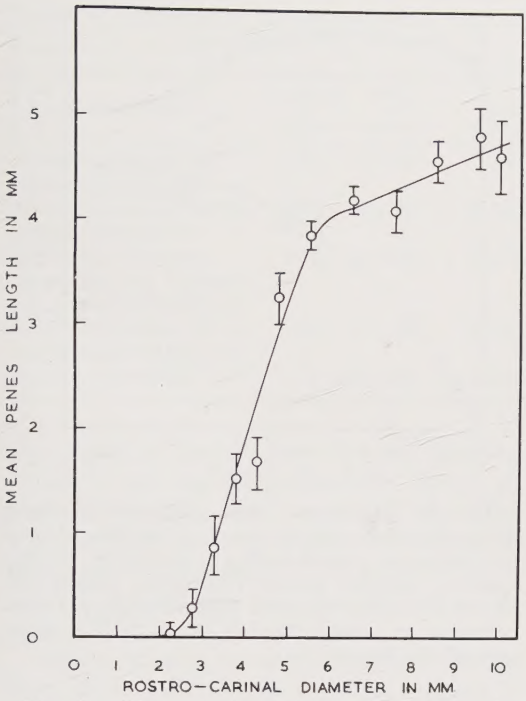


FIG. 5. The growth of the penis is shown by plotting the mean length of the penis for each size group. The standard error associated with each set of observations is marked by horizontal lines above and below each point. Heterogonic increase in length is evident in animals measuring 3 to 5 mm.

This was investigated by comparing three populations, A, B and C, in which growth took place at different rates. Population A was observed in the Menai Straits. The individuals were able to expand freely at the base and grew fast. Populations B and C were observed at Burnham-on-Crouch, where water temperatures were higher but greater population densities retarded growth very significantly. The population C was continually being smothered by new growth, which was not removed, and which led to many individuals showing a tendency to columnar growth. Table 2 shows that the onset of maturity was delayed considerably in the slower growing populations, but that growth in diameter was delayed even more. Hence the crowded populations eventually contained a higher proportion of ovigerous individuals in the smaller size groups. The percentage of individuals with egg masses in the larger groups, however, was less in the crowded population C than in the better nourished populations A and B.

In population C many of the specimens examined were columnar due to lateral pressure, hence the rostro-carinal diameter was not a satisfactory measure of size. They were not, however, as distorted as specimens of *B. balanoides* under comparable conditions. Despite this difficulty, there was no doubt that some of the mature specimens were smaller than any of the mature individuals of population A. The development of the male organs in very

TABLE 2. *Influence of crowding on development of ovaries*

Size range (mm)	Percentage of sample bearing egg masses in population		
	A	B	C
6–7	71	66	57
5–6	36	37	47
4–5	2	16	33
3–4	0	1	10
Max. size after 8 weeks' growth	8.3 mm	5.5 mm	4.2 mm
Date of first settlement	July 1–7	July 7–14	May 12–18
Date of first appearance of egg masses	Aug. 14–23	Sept. 21–28	Aug. 25–Sept. 7
Time to reach maturity	6–7 weeks	10–11 weeks	14–15 weeks
Temperature range of water during time to reach maturity	15–17°C	16.5–21°C	15–21°C

Population A On barge, Menai Straits, continuously immersed and growing freely at density of *ca* 1/cm². New spat removed.
B Intertidal, at L.W.N. Burnham-on-Crouch. *ca* 3/cm². New spat removed.
C Intertidal, at M.T.L. Burnham-on-Crouch. Very crowded with new spat allowed to accumulate over initial settlement. Density at first 30–50/cm², falling to 8–10/cm².

TABLE 3. *Influence of crowding on development of male organs*

Size range (mm)	Testis index°		Penis length	
	Normal population	Crowded population	Normal population	Crowded population
6–7	2.3	2.85	4.1	5.1
5–6	2.0	2.8	3.9	4.9
4–5	1.4	2.3	2.6	4.2
3–4	0.72	2.0	1.0	3.5
2–3	0.10	1.0	0.0	1.3

° Based on a scoring system
Well developed testis, vesiculae seminales full 3
Testes present, vesiculae seminales partially full 2
Trace of testis, vesiculae seminales not well developed 1
Male organs not seen 0

small individuals was also a feature of the more crowded population. It can be seen from Table 3 that individuals grown in a restricted space eventually develop testes at a smaller size and have larger penes than normal fast growing specimens of the same size.

Sexual maturity is thus seen to be mainly a function of size, but is also to a limited degree dependent on age. In our experiments on growth the specimens were given ample space competing organisms being removed at frequent intervals. The onset of maturity of the ovary would therefore be expected to correspond closely with that of population A (Table 2), the great majority of individuals beginning to produce broods at a size ranging from 5 to 7 mm in diameter. The testes would by this time be well developed and the penes have almost reached their maximum (contracted) length. Cross-fertilization at a distance of 2 cm would then be possible, since the fully extended penis can reach another individual lying at a distance of 3 to 5 cm (Crisp 1958).

INFLUENCE OF BREEDING ON GROWTH

Figure 6 represents the growth rates measured as the rostro-carinal diameter for the two series of barnacles, paired and isolated, averaged over all individuals.

Growth was approximately linear with respect to time until the barnacles reached a length of 5 to 7 mm, when it fell off. In this species, as in others, therefore, the decline of the growth rate from linearity corresponds closely with the onset of maturity

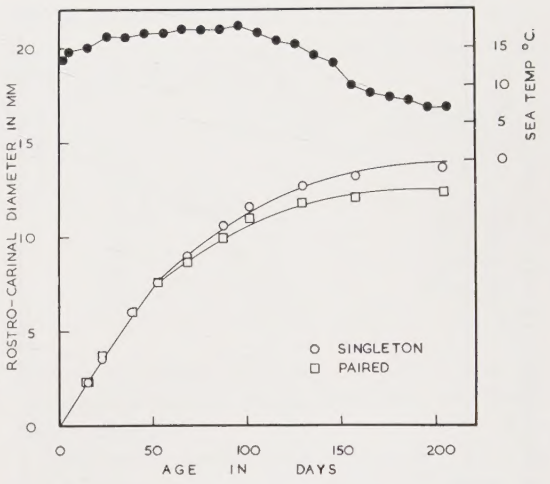


FIG. 6. The mean basal diameters measured along the rostro-carinal axis as a function of time for singletons (non-breeding), shown by circular symbols, and for paired (breeding) individuals, shown by square symbols. Mean weekly sea temperatures are shown by full circles in the upper part of the figure.

(Crisp 1960). Furthermore, Figure 6 shows that, although there was no difference between paired and isolated groups during the linear phase of growth, the paired specimens, which could breed, grew distinctly more slowly than the isolated specimens during the terminal phase of growth.

The results were confirmed statistically by treating the observations as two series; the first included observations up to 52 days after settlement, and the second, observations from 38 to 157 days. Observations were confined to the same 51 isolated and 46 paired individuals, which continued to grow for the whole period of the experiment, and observations on individuals which died were discarded.

In each treatment the best fitting regression line was obtained separately for the isolated and for the paired groups, and the mean square deviation about this regression was calculated. The observations were then pooled and fitted to a common regression line and the sums of squares associated with the difference between the two regression lines were obtained by subtraction. From the resulting analysis of variance shown in Table 4 it can be seen that over

TABLE 4. *Analysis of variance of linear part of growth curves*

Source of variation	Degrees of freedom	Sum of squares	Mean square
About regression for isolated individuals	202	97.193	0.481
About regression for paired individuals	182	64.531	0.355
Difference between regressions for isolated and paired individuals	2	0.004	0.002
About regression for all individuals	386	161.728	0.419
Due to regression	1	1561.546	1561.546

the period of growth from 0 to 52 days the isolated and paired individuals fitted almost exactly the same regression line. Deviations from the regression, due mainly to the slight non-linearity of the growth curve, were in fact greater than the difference between the two regression lines.

Before treating the second period, namely from 38 to 157 days, in a similar manner, it was necessary to make a transformation to correct the curve for non-linearity. The relationship found by trial to give the best correction was

$$l/(l_0 - l) = Kt \text{ where}$$
l_0 and K are constants.

This relation was capable of giving a fairly close fit over the whole curve of growth, but was fitted by the method of least squares only to the interval 38 to 157 days, during which the barnacles grew from about 6 to 13 mm. In using this expression the rostro-carinal diameter, l, was transformed to the parameter t/l to obtain a

TABLE 5. *Analysis of variance of later part of growth curves*

Source of variation	Degrees of freedom	Sum of squares	Mean square
About regression for isolated individuals	355	225.198	0.634
About regression for grouped individuals	320	215.058	0.672
Difference between regressions for isolated and grouped individuals	2	55.608	27.804
About regression for all individuals	677	495.864	0.732

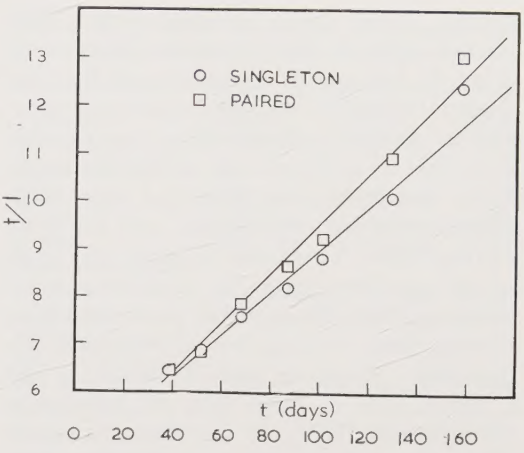


FIG. 7. Growth rate of single (circular symbols) and paired individuals (square symbols) transformed to the parameter t/l in order to obtain a closer fit to a linear regression.

linear regression of t/l on t for the paired and isolated sets of barnacles. As can be seen from Figure 7, the two lines obtained were different, and the difference between them was significantly greater than the variation of either paired or isolated individuals about their own regression (Table 5). Student's t test showed that the regressions differed significantly both in their slopes and in their means. The difference in the means showed that over the range studied the isolated specimens had reached a greater diameter. The difference in slope, $\Delta(t/l)/\Delta t$, implied that the isolated specimens were tending to reach a greater asymptotic diameter. The relevant equations fitting the two series were

$l_i/22.12 - l_i = 0.0100t$ for isolated specimens
$l_p/18.28 - l_p = 0.01335t$ for paired specimens

Clearly therefore the growth of paired specimens was retarded significantly from the point at which they began to produce broods, and this difference increased steadily as shown in Figure 6.

The difference between paired and isolated specimens will be ascribed to the fact that the former were able to breed, and so continually lost tissue in the form of egg masses. Another possible explanation of the difference was that paired specimens might have competed for the available food to a

greater extent than isolated ones. This seemed unlikely, since previous experiments using *B. balanoides* demonstrated that the growth in weight of paired specimens actually in contact did not differ significantly from that of isolated ones unless the population density was so high that other individuals crowded into contact with the pairs (Crisp 1960). It seemed unlikely that pairs of the smaller species, *E. modestus*, when separated by a distance that prevented their coming into contact, would compete appreciably. However, since *E. modestus* had a faster rate of beat than *B. balanoides* (Southward 1955), it was thought desirable to investigate this possibility in a subsidiary experiment.

GROWTH OF *ELMINIUS MODESTUS* IN COMPETITION WITH *BALANUS BALANOIDES*

An experiment was set up in 1960 with a pattern of pits similar to that shown in Figure 2, but with additional pits drilled 2 cm away from each of the originally isolated pits. Six of these pairs of pits were allowed to be occupied each by one cyprid of *B. balanoides* which settled just before *E. modestus*. The remaining single pits and pairs of pits were exposed to later settlement by *E. modestus*. The necessity to obtain a mixed settlement made it difficult to follow the previous pattern exactly, but it was possible to obtain about 6 pairs with mixed species and 6 pairs of *E. modestus* on each plate in a random arrangement. Thus it was possible, as before, to compare the amount of growth achieved by individuals of the latter species, which were paired and able to breed, with that of individuals which were unable to breed because they were paired with another species, *B. balanoides*. In *B. balanoides* the male organs were undeveloped. The individuals isolated from their own species were therefore in this experiment not growing separately but were in competition with much larger and faster growing specimens of *B. balanoides*.

Most of the pits were filled during the first 10 days or so; a few pits, however, were left unoccupied or the individuals in them were lost, so that they were not occupied by *Elminius* until a month had elapsed. These individuals, which were therefore a month younger than the rest, have been classified separately as Group 2 in Table 6 below.

TABLE 6. *Comparison of weight of individuals of* E. modestus *paired with their own species and with* B. balanoides

	Weight of isolated individuals (with *B. balanoides*)	Weight of paired individuals (with *E. modestus*)	Ratio isolated/ paired
Group 1. Mean age 130 days			
Number of specimens	39	42	
Wet weight	265.5 mg	230.8 mg	1.15
Dry weight	160.9 mg	134.3 mg	1.20
Shell weight	130.3 mg	109.7 mg	1.19
Tissue weight	30.6 mg	24.6 mg	1.24
Group 2. Mean age 100 days			
Number of specimens	6	6	
Wet weight	171.8 mg	153.8 mg	1.20
Dry weight	103.1 mg	88.8 mg	1.16
Shell weight	82.8 mg	70.8 mg	1.17
Tissue weight	20.3 mg	18.0 mg	1.13

Mean wet weight of *B. balanoides* = 919 mg
Mean dry weight of *B. balanoides* = 657 mg

The results show that, even though in competition with an individual of another species of about four times its weight, growth of the non-breeding individuals remained greater than that of breeding individuals. The difference, whether judged by wet weight, dry weight, shell weight or tissue weight, was significant at the 0.01 probability level. The difference was less than in the previous experiment carried out in 1959, but this is not surprising in view of the large size of the adjacent specimen of *B. balanoides* (Figure 1B) and the shorter growth period allowed before the specimens were weighed.

A small number of specimens of *B. balanoides* became isolated during the experiment on account of the loss of the adjacent *E. modestus*, while others were found to be paired inadvertently with a specimen of *B. balanoides* within a distance of 2 cm. It was possible therefore to use the data from these individuals to measure approximately the effect of competition between one specimen and another of *B. balanoides* under the

TABLE 7. *Comparison of isolated individuals of* B. balanoides *with individuals paired with their own species and with* Elminius modestus

Condition of barnacles	Number of specimens	Wet weight (mg)	Dry weight (mg)	Shell weight (mg)	Tissue weight (mg)
Isolated	4	969	696	605	91
Paired with *Elminius modestus*	44	916	655	566	89
Paired with *Balanus balanoides*	9	914	649	560	89

conditions of the experiment. The results are given in Table 7. Insofar as the small size of the sample allows conclusions to be drawn, it confirms that the influence on growth caused by competition is very small. The wet and dry weights of the barnacles were reduced by only 6%, while the tissue weight in paired specimens was only 2% less than in isolated ones.

We therefore conclude that the difference in weight observed between isolated and paired individuals could not have resulted from competition for food, but was mainly due to loss of tissue during reproduction.

GROWTH PARAMETERS OF *ELMINIUS MODESTUS*

Table 8 gives the weights of isolated and paired individuals at the end of the 1959 experiment when the barnacles were some 6 months old. It can be seen that the linear dimensions of the isolated specimens, which were prevented from breeding, were some 10% greater than those of the paired specimens, which bred normally. The volume, calculated from these measurements, was about 32% greater. The shell weights, as expected, showed a similar increment of 33%, but the total weight was about 40% greater in isolated specimens. This discrepancy appeared to be due to the greater tissue weight, especially when dried, in the isolated specimens. Such a discrepancy was not wholly unexpected, for after removing the barnacles from the panels we were struck by the dense tissues of the ovary, which often appeared to have a two-layered structure and seemed to fill completely the mantle space of all the isolated specimens.

An interesting feature of the parameters shown in Table 3 was the fact that the

TABLE 8. *Growth parameters of isolated and paired individuals of* Elminius modestus

Dimension	Mean for isolated individuals	Mean for paired individuals	Ratio paired/isolated
Rostro-carinal diameter	13.553 mm	12.318 mm	1.100
Breadth	13.046 mm	11.858 mm	1.100
Height	4.652 mm	4.171 mm	1.115
Opercular valves:			
length	5.904 mm	5.781 mm	1.021
breadth	4.492 mm	4.252 mm	1.056
Volume of truncated cone equivalent to external dimensions of barnacle	331.62 mm^3	251.20 mm^3	1.320
Total weight:			
wet	315.80 mg	220.96 mg	1.429
dry	211.89 mg	151.04 mg	1.403
Tissue weight:			
wet	137.30 mg	86.96 mg	1.579
dry	33.39 mg	17.04 mg	1.960
Shell weight (dry)	178.50 mg	134.00 mg	1.332
Opercular valves	16.747 mg	14.833 mg	1.129
Carina	38.854 mg	28.674 mg	1.355
R. lateral	38.798 mg	28.432 mg	1.365
L. lateral	37.489 mg	27.664 mg	1.355
Rostrum	46.610 mg	34.616 mg	1.346

growth of the opercular valves was less affected by breeding than that of other structures. Whereas the shell compartments of isolated specimens showed a 10 to 12% linear increment, the valves showed an increment of only 2 to 5%. Correspondingly, the weights of the compartments of isolated specimens were 35% higher, but those of the valves were only 13% higher. We have noticed that in crammed columnar individuals of many species the valves usually remain relatively large, whereas in flattened fast spreading individuals they are relatively small. The growth of the valves therefore appears to be less dependent upon environmental factors than that of other parts of the shell.

LOSS OF TISSUE WEIGHT DUE TO BREEDING

In the 1959 experiment the dry weight of the egg masses averaged over all paired specimens containing them was 4.131 mg. On the assumption that breeding begins at an age of 40 days when the rostro-carinal diameter has reached 6 mm, there would

be time from mid-July until December for about 8 to 10 broods. This figure was calculated from the temperature of the sea during this period and the relationship between the brood period and temperature given by Crisp and Davies (1955).

Since the size of the egg mass is likely to be proportional to volume and therefore to the cube of the rostro-carinal diameter, the total weight of eggs produced will be less than the product of 4.131 and the number of broods, since 4.131 is the weight of the last and largest brood. On the assumption that the broods are produced at equal intervals of linear growth, and their weight is proportional to the body volume, we may calculate for 8 broods a total weight of 15.9 mg and for 10 broods a weight of 20.1 mg. The difference in the dry weight between isolated and breeding specimens was $211.9 - 151.0 = 60.9$ mg, but of this 44.5 mg was accounted for by the difference in the mean dry weights of the shell. Hence the difference in mean tissue weight of the breeding and non-breeding specimens was 16.4 mg. To obtain a comparison of tissue weights of isolated and paired specimens, excluding the weight of embryos in the latter, the weight of one pair of egg masses should be subtracted from the mean weight of the paired specimens, since the dry weight of most of them included that of the egg masses. After making this small correction the average loss in tissue weight due to breeding was 20.5 mg. The close agreement of this figure with the estimated output of tissue in the form of egg masses indicates that ovarian regeneration is the main factor reducing the reserves of breeding specimens so causing them to grow more slowly. The loss of seminal fluid may either be a negligible drain on resources or be a common factor to both breeding and non-breeding specimens.

The curves of growth in Figure 4 show that a decline in growth rate occurs both in breeding and non-breeding specimens and this decline begins before the sea temperatures fall appreciably from their maximum. The decline in growth, coincident with the onset of maturity, cannot therefore be ascribed either to seasonal changes in temperature nor to loss of tissue as a result of breeding. Nor is it likely to be due to changes in nutritional content of the water, since similar growth curves may be obtained for specimens which settle in late autumn and reach maturity in early spring, and also for those that settle in spring and reach maturity in early summer.

CONTROL OF GROWTH OF SHELL AND TISSUE

The shell of non-breeding specimens was considerably larger than that of breeding individuals, the increase corresponding closely with the greater weight of body tissue. The weight of the shell was always closely related to that of the tissues, though it must be remembered that in this investigation all the individuals were of roughly the same age and had grown under the same conditions. It is possible that variations in the relative weights of shell and tissue may exist which depend upon the age of the individual and on the environment in which it grew.

It has been shown (Crisp 1960) that, if the collection of food by the cirri was the limiting factor in growth, and if the cirral net grew isometrically with the rest of the barnacle, then the relation between the diameter of the base and time would be linear. Though linearity is typical of the early part of the growth curve of many species, it does not necessarily follow that food is the limiting factor; such a view may be an oversimplification. Nevertheless, growth of a barnacle is greatly dependent on the water flow past it, and therefore probably on the amount of nutrient matter carried to it by the water (Crisp 1960). If we accept that growth rate is largely dependent on the food supply it follows that after maturity when, even in non-breeding individuals, growth slows down, another limitation must superimpose itself on that due to the amount of food collected. An obvious suggestion is that changes associated with maturity occur in the endocrine balance, and that these changes cause growth to be reduced below the rate that purely nutritional factors could support. However, our results show

that even if the eggs as well as body tissue are taken into account, the mean rate of tissue production of the smaller breeding individuals is no more than that of the large non-breeding ones. If growth in body size were being controlled at a level within the capacity of the animal's nutritional potential, it seems likely that the breeding specimens would have been able to make good, in part at least, their losses caused by oviposition. It seems probable, therefore that, even during the period of declining growth rate, the rate of tissue production was limited absolutely by some factor such as food collection, rate of digestion of food, or mobilization of tissue-building material.

We shall therefore consider mechanisms that might lead to a fall in growth rate and at the same time be consistent with a strict limitation on the amount of material available for tissue production. The simplest suggestion would be to postulate that, when the animal reaches a certain size, roughly coincident with maturity, growth ceases to be isometric because some structure, vital to food collection or utilization, such as the cirral net or gut, grows at a rate that is less than proportional to that of the rest of the animal. Another and perhaps more likely explanation depends on the physical limitation caused by diffusion. If growth continued isometrically, diffusion processes could not keep in scale with increasing size, but would cause the rate of transport of metabolites to lag behind. Fick's law states that for a given fall in concentration across, say, a membrane, the transport of mass will be proportional to the area and inversely proportional to the thickness. Hence, if the volume increase of the animal were limited by diffusion of mass, then, assuming isometric growth, its rate of volume increase dv/dt would be proportional to an area ÷ a thickness and hence to the first power of a characteristic length l; the rate of increase in length dl/dt will then vary at $1/l$. Such processes would become increasingly important with increase in size and the diameter would follow the square root of the animal's age and not increase directly with it.

Further work to elucidate the fundamental processes controlling growth in cirripedes is clearly desirable. Most of the problems that have been discussed above, such as the food supply and physico-chemical factors, require a greater degree of control than is possible in the field. They depend therefore for their solution on developing laboratory techniques for feeding and maintaining these animals in a controlled environment.

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THE DETERMINATION OF ZERO-THICKNESS ACTIVITY IN GEIGER COUNTING OF C^{14} SOLUTIONS USED IN MARINE PRODUCTIVITY STUDIES

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ABSTRACT

Liquid scintillation counting methods are described for the determination, with a precision of ± 2%, of the absolute activities of C^{14} stock solutions to be used in marine productivity studies. The efficiency of a windowless Geiger counter was found to be 58% by counting very thin films of labelled plastic whose absolute activities were then determined by liquid scintillation counting. The zero-thickness Geiger activities of several C^{14} stock solutions were determined with a precision of ± 3%, and an accuracy of ± 5%. This method was compared with the method of extrapolating self-absorption curves of $BaC^{14}O_3$ planchets of varying thicknesses. It was found that the self-absorption curve between 0.5 and 6.0 mg/cm^2 conformed equally well to an exponential as to a hyperbolic function. Extrapolation of these functions to 0 mg/cm^2 gave zero-thickness activities 24% and 17% respectively lower than those obtained by the new method described, showing that the self-absorption curve varied considerably from both the exponential and hyperbolic function at thicknesses below 0.5 mg/cm^2. This is attributed largely to back-scattering. Empirical self-absorption curves were found to be unsatisfactory due to the impossibility of preparing planchets of $BaCO_3$ in the critical range of 0 to 0.5 mg/cm^2.

INTRODUCTION

In the measurements of primary production of organic matter by marine phytoplankton by the method originally proposed by Steemann Nielsen (1952), solutions of $NaHC^{14}O_3$ are mixed with samples of sea water containing phytoplankton. After suitable incubation the phytoplankton are filtered off and the amount of $C^{14}O_2$ taken up by the organisms is measured by counting under a Geiger counter, usually of the windowless type. As the amount of phytoplankton is usually very small, it is assumed that there is no self-absorption of the β-particles under these counting conditions. In order to calculate the rate of uptake of the $NaHC^{14}O_3$, it is necessary to know the amount of activity added. This activity must be measured under the same counting conditions as those of the plankton samples, *i.e.*, with the same counting geometry and with no self-absorption of the β-particles.

The self-absorption of C^{14} β-particles has been extensively studied by Calvin *et al.* (1949) and Yankwich *et al.* (1947), chiefly by the preparation of self-absorption curves using planchets with varying thicknesses of $BaC^{14}O_3$. Zero-thickness activity can then be estimated by extrapolating the self-absorption curve.

Various modifications of this principle have been used (Steemann Nielsen 1952, Doty 1956, Doty 1959, Thomas 1959). However all of these suffer from the difficulty, if not impossibility, of preparing sufficiently thin planchets, which are necessary if the extrapolation to zero-thickness is to be reliable. Jitts (1957) has tried to avoid this difficulty by assuming that self-absorption is exponential and therefore making the extrapolation mathematically from planchets of suitable thickness. This can be criticized on the evidence that self-absorption is not strictly exponential.

In the work reported here, a method is described for the reliable preparation of planchets down to a thickness of 0.5 mg $BaC^{14}O_3$/cm^2. The extrapolation of the self-absorption curve thus obtained is made in various ways.

In order to avoid the difficulties in the extrapolation of self-absorption curves, a new method is described for the determination of zero-thickness activity. In this, the latter is calculated from the absolute activity of the C^{14} solution and the efficiency of the windowless Geiger counter.

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METHODS

The preparation of $BaC^{14}O_3$ planchets

Planchets of $BaCO_3$ varying from 0.5 to 6.0 mg/cm^2 each containing the same amount of C^{14} activity were prepared in triplicate from three separate 1-ml ampoules of each C^{14} stock solution.

Each ampoule was diluted to 500 ml with a solution containing 1.36 g of Na_2CO_3 per liter of CO_2 free distilled water; 0.5 ml aliquots of this diluted C^{14} solution were pipetted into seven 30-ml conical flasks containing 0, 0.5, 1.5, 2.5, 3.5, 4.5, and 5.5 ml respectively of the same Na_2CO_3 solution used in diluting the ampoules.

These flasks were first treated with "Desikote" (Beckman Instruments, U.S.A.), a surface active silicone product, to allow complete transference of their contents without washing. To these flasks 0.05, 0.1, 0.2, 0.3, 0.4, 0.5, and 0.6 ml of 6.26%. $BaCl_2$ were then added.

The precipitates of $BaCO_3$ thus formed were allowed to stand for two hours with gentle swirling every half hour. Planchets were then prepared with each of these precipitates by their total transfer to tared membrane filters ("Millipore" Type HA, Millipore Corporation, U.S.A., 15/16 in. diameter, effective pore size 0.5μ) using an apparatus with a filtration area of 2.5 cm^2. The planchets were sucked dry without washing and then placed in a desiccator over silica gel for 24 hr, when they were weighed and their activity determined.

The preparation of thin films of plastic labelled with C^{14}

A plastic labelled with C^{14} ("Perspex," polymethyl methacrylate, Amersham, England, Type CFP3) was dissolved in redistilled acetone to give a solution containing 26 mg/ml. This solution was dropped from a glass rod on to distilled water in a 10-cm Petri dish. The plastic formed a thin film on the surface of the water.

A portion of this film was made to cover the surface of a millipore filter (15/16 in. diameter) by placing the filter with a paper backing sheet under the water surface before forming the film. The filter was then carefully lifted out under the film so that a uniform layer of plastic was caught on the surface of the filter. The backing sheet was then removed, thus ensuring that no plastic was caught on the underside of the filter.

After drying in a desiccator, the filter was cut to give a disc of 2.5 cm^2, the same as the area of $BaC^{14}O_3$ or labelled phytoplankton in planchets prepared by filtration. This disc was placed on a planchet holder with an annular ring of clean millipore filter around it, thus ensuring the same conditions of geometry and back-scattering for Geiger counting as for other planchets of phytoplankton.

Films prepared in this way were less than one micron thick. Their Geiger activity varied from 150 to 1,000 cpm.

The measurement of Geiger activity

The Geiger activities of all planchets were measured with a windowless gas flow counter made in this laboratory and resembling the Tracerlab, U.S.A., SC-16 counter. The Geiger gas used was Tracerlab Q gas, and the scaler was an EKCO N530.

All planchets were counted to 10,000 counts, giving a probable counting error of 1%. Activities were corrected for background and standardized to a C^{14} reference source (2.5 cm^2 of Amersham CFP2 labelled "Perspex") to correct for any instability in the counter.

Liquid scintillation counting of C^{14} solutions

1st Series.—This series was carried out at the Donner Laboratories, University of California, Berkeley, U.S.A.

Liquid scintillation counting was used to determine the absolute activities of four solutions of $NaHC^{14}O_3$ using duplicate 1-ml ampoules of each solution. The contents of each ampoule were transferred to a standard flask containing about 10 mg of Na_2CO_3 and diluted with H_2O to 100 ml.

Four 100 μl aliquots were pipetted from each flask into scintillation tubes containing 10 ml of a phosphor of the following compositions:

5 volumes toluene
5 volumes para-dioxane
3 volumes ethanol
50 g/L napthalene
4-5 g/L P.P.O. (2-5 diphenyl oxazole)
100 mg/L P.O.P.O.P. (phenyl-ozazole-phenyl-oxazole-phenyl)

To two of these four tubes was also added 100 μl of standard C^{14} labelled toluene with an absolute activity of 14×10^3 dpm/100 μl. A tube with 10 ml of phosphor but with no added activity was also prepared to measure background. These samples were placed in a Packard Tri-Carb Automatic Counter and each counted 5 times over a period of 2½ days.

The counts of each sample were corrected for background by subtracting the average of the 5 readings of the background sample from each of the readings of the other samples. The efficiency of counting each sample was calculated by subtracting the activity of the sample from that of the sample plus standard, and dividing the result by the absolute activity of the standard (14×10^3 dpm). The absolute activity of each sample of C^{14} stock was then calculated by dividing its counted activity by the corresponding efficiency in each run. The absolute activity of each ampoule was then calculated by multiplying the mean activity by the dilution factor of 1,000.

2nd Series.—This series was carried out at the John Curtin School of Medical Research, Australian National University, Canberra, Australia. A Packard Tri-carb manual counter was used to determine the absolute activities of 8 C^{14} solutions, with a scintillator composed of:

4 g PPO
100 mg POPOP
900 ml toluene
100 ml ethanol

The contents of 1-ml ampoules of the stocks were transferred to flasks containing 10 mg $NaHCO_3$ and made up to 100 ml with H_2O. Duplicate 20 μl aliquots of each of these dilute solutions were pipetted into vials containing 10 ml of the above scintillator. The vials were left standing in a deep-freeze for one hour, then the activity of each was counted twice for 10 min.

As no internal standard was available, the efficiency of this counting system was estimated by comparing the count rates of three of the solutions with their absolute activities which had been determined in the first series. The mean of the three efficiencies thus determined was used to calculate the absolute activities of the 8 solutions.

Liquid scintillation counting of the labelled plastic films

The thin plastic films mounted on millipore filters were dissolved in counting vials which contained 10 ml of a scintillator consisting of 4g PPO and 100 mg POPOP dissolved in one liter of toluene. Whilst the millipore filters were insoluble in the scintillator, they became transparent and settled to the bottom of the vials. The small amounts of plastic in the films were readily soluble. The vials were left standing overnight, then each was counted three times for 10 min with a Packard Tri-carb manual counter.

To each of these vials was added 100 μl of a C^{14} labelled benzoic acid solution in some of the same scintillator described above. The absolute activity of this benzoic acid solution had been previously determined against C.S.I.R.O. C^{14} stock No. 6 as 2453 dpm/100 μl. The activty of each vial was then counted for 10 min. From the

TABLE 1. *The efficiency of a windowless Geiger counter calculated from the Geiger and absolute activities of thin C^{14} labelled plastic films*

Perspex film number	Geiger activity (cpm)	Absolute activity (dpm)	Geiger efficiency (%)
13	277	478	57.9
14	538	880	61.1
15	275	520	52.9
16	675	1208	55.9
17	212	330	64.2
18	284	494	57.5
19	190	364	52.2
20	169	279	60.6
21	197	336	58.6
22	333	574	58.0
23	337	604	55.8
24	267	443	60.3
			Mean: 57.9

increase in the activity of each vial the efficiency was calculated. Multiplying the mean activity of each perspex film by this efficiency gave the absolute activity of the film.

RESULTS

The efficiency of a windowless Geiger counter

Twelve films of C^{14} labelled plastic were prepared, and their Geiger activity was measured with the windowless Geiger counter. Their absolute activity was determined by liquid scintillation counting. The ratio of the Geiger activity to the absolute activity of each film gave the efficiency of the Geiger counter. The results (Table 1)

TABLE 2. *The absolute activities of several C^{14} solutions determined by liquid scintillation counting*

C^{14} stock number		Absolute activities (dpm per ampoule ÷ 10^6) 1st count	2nd count	3rd count	4th count	5th count	Mean	Mean each stock
Hawaii 5 1st ampoule	A[3]	7.08	7.19[1]	7.08	7.07	7.06	7.10	6.94
	B	6.89	7.01	6.99	7.03	7.04	6.99	
Hawaii 5 2nd ampoule	A	6.79	6.82	6.84	6.77	6.84	6.81	
	B	6.80	6.92	6.85	6.86	6.84	6.85	
Hawaii 6 1st ampoule	A	8.45	8.50	8.52	8.51	8.45	8.49	8.39
	B	8.19	8.17	8.14	8.17	8.09	8.15	
Hawaii 6 2nd ampoule	A	8.52	8.55	8.63	8.59	7.76[1]	8.41	
	B	8.47	8.48	8.60	8.52	8.44	8.50	
Hawaii 7 1st ampoule	A	7.96	8.04	8.01	7.99	7.99	8.00	7.97
	B	7.94	7.94	7.92	7.94	7.91	7.93	
Hawaii 7 2nd ampoule	A	7.99	8.01	7.99	7.98	7.99	7.99	
	B	7.95	7.96	7.95	7.96	8.00	7.96	
CSIRO 6 1st ampoule	A	15.87	15.87	15.84	15.77	15.89	15.85	15.84
	B	15.35	15.11	14.29	13.18	11.96	—[2]	
CSIRO 6 2nd ampoule	A	15.97	15.98	16.01	15.07[1]	15.93	15.79	
	B	15.84	15.88	15.91	15.91	15.82	15.87	

[1] Doubtful figures, but included in mean
[2] This sample not included in mean
[3] A and B are aliquots from each ampoule

TABLE 3. *The efficiency of the liquid scintillation counting system*

C^{14} stock number	Absolute activity ($\div 10^6$ dpm/ml)	Scintillation activity ($\div 10^6$ cpm/ml)	Efficiency (%)
Hawaii 5	6.94	2.62	37.8
Hawaii 7	7.97	2.99	37.5
CSIRO 6	15.84	5.87	37.1
		Mean:	37.4

varied from 52% to 64%. The mean of these gave the efficiency of the Geiger counter as 58% ± 3 (standard error).

The zero-thickness activity of several C^{14} stock solutions

Liquid scintillation counting (1st Series) was used to determine the absolute activities of Hawaii C^{14} stock numbers 5, 6, and 7, and C.S.I.R.O. C^{14} stock number 6. The results are given in Table 2. The variations of replicate counts on the same sample and of replicate samples of the same stock showed the mean activities for each stock to have a precision of better than ± 2%.

In the second series the absolute activities of Hawaii C^{14} stock numbers 5 and 7, and C.S.I.R.O. C^{14} stock numbers 2, 3, 4, 5, 6, and 7 were determined. The efficiency of the counting system used in this second series was determined by comparing the activities found here and in the first series described above for Hawaii C^{14} stock numbers 5 and 7, and for C.S.I.R.O. stock number 6. This comparison (Table 3) gave a mean efficiency for the system of 37.4%.

In this second series, the absolute activities of the C^{14} stock solutions were calculated from their scintillation activities and the efficiency of the scintillation counting system. In the preceding section, the efficiency of the windowless Geiger counter had been determined as 58%. By applying this factor to their absolute activities, the zero-thickness Geiger activities of the C^{14} stock solutions were calculated. The results are given in Table 4.

The self-absorption of $BaC^{14}O_3$ planchets

Planchets of $BaC^{14}O_3$ were prepared in three separate series, twice with C.S.I.R.O. C^{14} stock number 6 and once with C.S.I.R.O. C^{14} stock number 7. Their activities were measured with the windowless Geiger counter. In Figures 1, 2, and 3 the activities of the planchets in each series are plotted as both log activities and reciprocal activities against their respective weights.

TABLE 4. *The zero-thickness Geiger activities of several C^{14} stock solutions, calculated from their absolute activities and the efficiency of the Geiger counter*

C^{14} stock number		Scintillation activity ($\div$ 5,000 cpm/ml) 1st count	Scintillation activity ($\div$ 5,000 cpm/ml) 2nd count	Mean absolute activity ($\div 10^6$ dpm/ml)	Geiger activity ($\div 10^6$ cpm/ml)
Hawaii 7	i)	606	608	8.00	4.64
	ii)	602	572		
Hawaii 5	i)	517	529	6.99	4.05
	ii)	525	521		
CSIRO 2	i)	3367	3438	46.33	26.86
	ii)	3510	3550		
CSIRO 3	i)	1395	1418	18.39	10.66
	ii)	1336	1352		
CSIRO 4	i)	1249	1278	16.72	9.72
	ii)	1225	1251		
CSIRO 5	i)	109	101	1.62	0.94
	ii)	136	139		
CSIRO 6	i)	1169	1174	15.70	9.10
	ii)	1177	1177		
CSIRO 7	i)	1339	1351	17.92	10.39
	ii)	1335	1342		

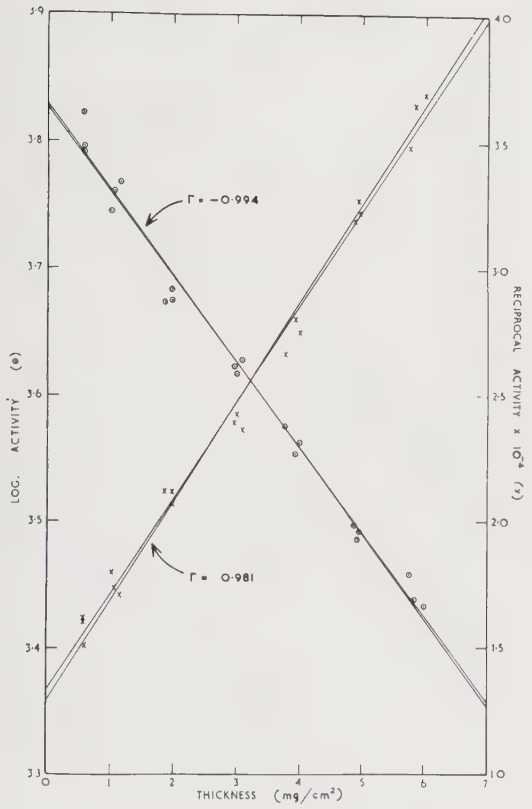

FIG. 1. The apparent activites of a series of planchets prepared from C.S.I.R.O. C^{14} stock No. 6, plotted as both logarithms and as reciprocals, and showing the linear regression lines and their associated coefficients of correlation.

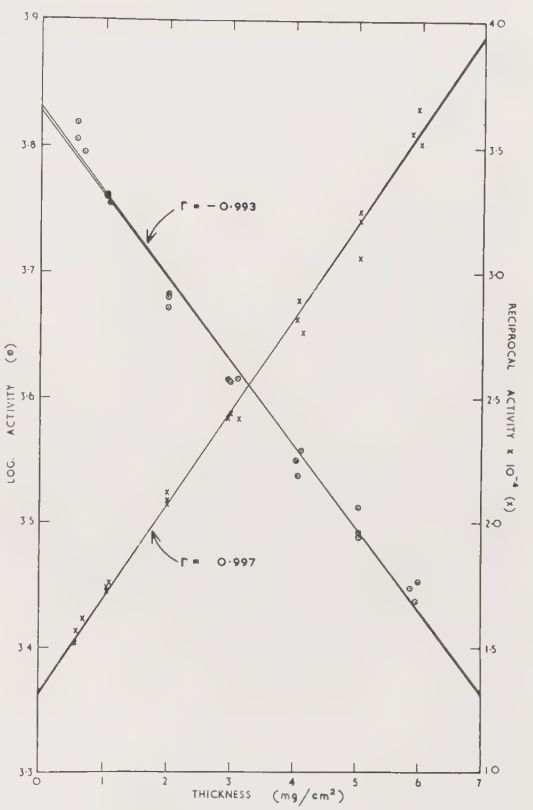

FIG. 2. The activities of a second preparation of planchets from C.S.I.R.O. C^{14} stock No. 6.

In each case the linear regression lines and their correlation coefficients for both the log activities and the reciprocal activities were calculated and the lines extrapolated to zero-thickness. In this way, estimates were calculated for the zero-thickness activities of the C^{14} stock solutions by both exponential and hyperbolic extrapolation of the experimental data for self-absorption. These estimates expressed as percentages of the zero-thickness activities of the C^{14} stock solutions are given in Table 5.

DISCUSSION

The liquid scintillation counting techniques measured the absolute activities of C^{14} solutions with a precision better than ± 2%. The counting efficiency of a windowless Geiger counter was measured to a precision of ± 3%. This means that the method suggested is able to determine the zero-thickness activity of the solutions with a precision of about ± 3%. However, it must be remembered that all the scintillation counting measurements were made by reference to the internal C^{14} standard toluene used in the first series of scintillation counting. As it is not possible to obtain C^{14} standards with an accuracy of much better than ± 5%, the determinations of zero-thickness activity whilst precise to ± 3% cannot be claimed to be accurate to more than ± 5%.

The technique itself is quite simple, certainly less subject to manipulative difficulties than the preparation of $BaC^{14}O_3$ planchets. The efficiency of the Geiger counter need be determined only once, if the Geiger counts are thereafter always corrected to a permanent C^{14} reference

TABLE 5. *The zero-thickness activity of C^{14} solutions calculated by three different methods*

C^{14} stock number	Zero-thickness activity					
	Calculated from absolute activity		Exponential extrapolation		Hyperbolic extrapolation	
	(÷ 10^6 cpm/ml)	%	(÷ 10^6 cpm/ml)	%	(÷ 10^6 cpm/ml)	%
CSIRO 6	9.10	100	6.75	74.2	7.57	83.2
CSIRO 6	9.10	100	6.72	73.9	7.52	82.6
CSIRO 7	10.39	100	7.59	73.1	8.62	83.0
			Mean:	73.7	Mean:	82.9

source such as a labelled plastic. This is in any case necessary as windowless Geiger counters are usually found to be unstable.

It has been shown that it is possible to obtain suitable $BaC^{14}O_3$ planchets containing between 0.5 mg and 6.0 mg $BaCO_3/cm^2$.

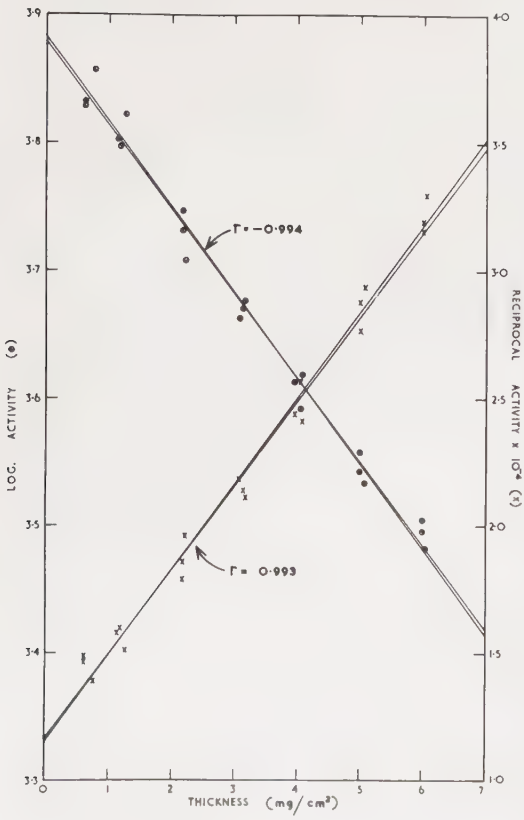

FIG. 3. The activities of a series of planchets prepared from C.S.I.R.O. C^{14} stock No. 7.

If it is assumed that self-absorption is exponential (as in Jitts 1957) or is hyperbolic (as in Hendler 1959), then the data can be extrapolated to zero-thickness. However, whilst the zero-thickness activities obtained in these ways can have a precision of the same order as the above, their values have been shown to be low by 26% and 17% respectively (Table 5). The correlation coefficients between thickness and apparent activity were highly significant (0.01% level) when self-absorption was treated either as a hyperbolic or as an exponential function, although an inspection of Figures 1, 2, and 3 does suggest that there is still some curvature in both cases. These results show that the self-absorption curve between 0.5 and 6.0 mg/cm² conforms equally well with both the exponential and the hyperbolic function, but varies considerably from both below 0.5 mg/cm². It must also be stressed that this is only the case for the windowless counter under study.

Even neglecting the disadvantages of the subjective errors involved, any attempt to draw self-absorption curves through the experimental points could not materially improve the extrapolation to zero-thickness unless more points were obtained in the critical range between 0 and 0.5 mg/cm². Thomas (1959) reported having prepared planchets of less than 0.1 mg $BaCO_3/cm^2$. However, if it is assumed that the $BaCO_3$ particles are 1μ in diameter and that these particles formed a mono-particulate layer completely covering the planchet, then the

density of $BaCO_3$ of 4.4 g/cm^3 would make the thickness 0.4 mg/cm^2. It follows that planchets with thicknesses between 0 and 0.5 mg/cm^2 could only be of particles which do not completely cover the filter. In this case the self-absorption would remain constant through the range 0 to 0.5 mg/cm^2 and any change in the apparent specific activity of the $BaCO_3$ would be due to changes in geometry and back-scattering.

In these studies the efficiency of the windowless counter was found to be 58%. As the geometry could not be greater than 2π and if this efficiency is correct, then at least 8% of the β-particles counted must have been back-scattered. No detailed study has been published on the β efficiency of a windowless Geiger counter, but various figures have been quoted for this efficiency from 40% (Thomas 1959) to 55% (Tracerlab catalogues). If in the figures published by Thomas (1959) it is assumed that self-absorption remained constant in his planchets of less than 0.5 mg $BaCO_3$/cm^2, back-scattering can be estimated to cause a 20% increase in the activity at 0 mg/cm^2 over that at 0.5 mg/cm^2. These considerations show that in the Geiger counting of thin planchets of C^{14} labelled material as much consideration should be given to back-scattering conditions as to self-absorption.

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COMMUNICATION OF STRUCTURE IN PLANKTONIC POPULATIONS[1]

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ABSTRACT

Two alternative approaches are usual in the study of ecosystems. One emphasizes biomass and production and deals with the system in terms of matter and energy; the other is more concerned with structure. Both approaches are complementary and for their synthesis we need quantitative expressions for structure and for structure transmission along time. Information theory can be helpful. Biotic diversity is an expression of the capacity for carrying information of the ecosystem (= "channel width") at the level of individuals. Diversity can be appreciated also at other levels, *e.g.*, at the biochemical level (pigment composition of phytoplankton). Many factors, in planktonic communities chiefly turbulence of water, introduce an amount of noise. Ecosystems can accumulate "history" or increase maturity—succession—only if "noise" remains relatively low. As changes in the energy flow across the ecosystem are related to diversity, this is an area where the study of relations between mass, energy and structure may be rewarding.

The scientific study of assemblages of organisms present in natural communities has been conducted in two different and separate ways. In the first, which is awakening active interest today, one considers assemblages in terms of matter (biomass) and energy flow (productivity) and is concerned with the forces that introduce changes in the system (true dynamics). In the second and more traditional approach, one analyzes the community as a complex of individuals belonging to different species with definite ecological requirements; changes are dealt with by so-called population dynamics, or, more correctly a population kinematics. Both approaches have developed with a certain independence and a large gap has been growing between them. To bridge this important gap seems to be a rewarding field of ecological research.

Lack of coordination between similar complementary aspects, one concerned with matter and energy and the other with structure, causes difficulties in many other areas of biology also. For instance in the speculations on the origin of life, the biochemical hypotheses are ingenious but are untenable until provision is made for a mechanism for isolating the products of reactions through some sort of spatial structure of the system. How did the primeval "soup" develop an organization?

In living systems spatial configurations may influence the future, but time is required to build an organization, accumulate biomass, and canalize energy. Just as there are rate limitations for construction of a living being through evolution, so the slower velocities at which organisms grow, travel, multiply, act, react, make tries, and select possibilities limit rates for evolution of ecological structure.

It is proposed that the speed at which organization can be built and transmitted involves specific problems of communication theory. The "information" in an ecological set may be measured (Margalef 1957). This note concerns the time change of informational content of ecological structure during the exercise of decisions or selection that influence future events, building up more organization in this or that way, and channelling energy along certain paths.

The static aspect of the structure: Biotic diversity or channel capacity

Ideas developed in the field of information theory prove helpful for developing quantitative expressions of structural properties of ecosystems. So far, the application of information theory to ecological prob-

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lems has been unimaginative and hardly qualifies as information theory or theory of communication. We have contented ourselves with expressing the degree of complexity of a biotic community as a function of the number of distinguishable states that can be assumed by a given set of individuals.

If N_a, N_b . . . N_s are the numbers of individuals of species *a*, *b*, . . . *s*, and N is their sum, the expression for information

$$I = \frac{1}{N} \log_2 \frac{N!}{N_a!\, N_b! \ldots N_s!} \qquad (1)$$

represents an entropy and proves an advantageous substitute for the proposed indices of "biotic diversity." I is expressed in average number of bits per individual.

The actual structure of a population at a given time (t_1) represents a complex pattern. For the moment, consider that different individuals of the same species are indistinguishable and interchangeable. Such simplification discards a great mass of "information" of finer quality and amounts to the adoption of a *code*, for building a model that may be convenient in our preliminary approach. The biotic diversity (1) is a measure of the entropy of the system after coding and in an actual situation at time t_1 is the maximum amount of information.

The dynamic aspect of structure: Organization or integration

Communication theory concerns a certain amount of coded information put into a channel, the way it flows along the channel becoming more or less deteriorated through noise, and how any remaining information is recovered and decoded to be put into operation or use.

The initial structure of the community at time t_1 may also be considered as a message in the simple way described above. The comparison of species with the different letters or symbols of an alphabet is here pertinent. At a later time (t_2) the structure of the same community may be equal or different, but is interpretable in the terms of the same *code*. We can construct a model based on information theory to describe how populations change along time. However we have to remember that we are dealing only with a small fraction of the total amount of organization or "information": the limitation is fixed by our coding.

The diagram in Figure 1 indicates the coding and transmission of information in a mathematical or analogue electrical circuit parallel to the transmissions of information in the real ecological association.

Changes, if any, can effect: 1) the capacity of the channel, and 2) the degree of effective utilization of such capacity. Biotic diversity measures the maximum information that can be transmitted and, thus, the capacity of the channel. Biotic diversity rides on a "carrying wave" of births and deaths. The relative constancy in the proportions of the different species, that is a requisite for the conservation of channel capacity, has to be kept by negative or stabilizing feedback. In fact, the channel can widen or contract with time, owing to extinction of species, to introduction of new ones, or to differential multiplication of those already present.

It is customary to assign those species between which there is a negative feedback to separate ecological niches. Preservation of biotic diversity is, thus, related to the niche structure of the community. As the number of niches increases along succession up to a certain level, the carrying capacity of the channel widens. But the part of biotic diversity contributed by the possible existence of more than one species in a niche, drops as this event becomes less probable. Actually, with succession, biotic diversity can drop, as a result of the combination of both trends. Information carrying capacity of the channel, in terms of the selected code, can thus be reduced. But we have to remember that such changes are accompanied by the substitution of species by competition, and by a shift of "information" from the interindividual structure to the intraindividual level, where it is no longer apparent in our model.

Imagine that in the time elapsed from t_1 to t_2 no changes have occurred in the localization of species and that there has been an exact replacement of dead individ-

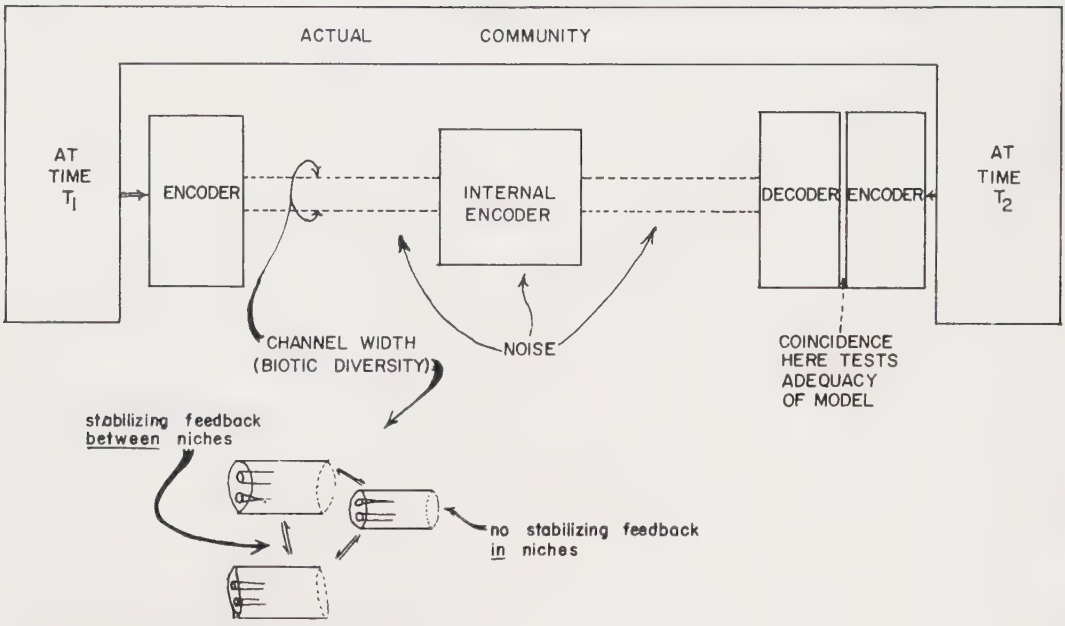

FIG. 1. Parallel between information transmission in the ecological community and in an analogue circuit.

uals. The pattern has been preserved and the information I_1 at time t_1 is completely recovered, then $I_2 = I_1$ at time t_2. Such may be actually the case in a community fixed on a solid substrate, but also in a community in which organisms have moved in the meantime in a regular and predictable way, so that their changes of place can be considered as a strictly determined encoding.

Ordinarily, a number of ecological factors (in planktonic communities mainly the turbulence of water) introduce randomness in the system or noise in terms of communication theory. Amount of noise, is highly relevant to the information to be transmitted. If the information contained in an initial pattern as gained through historical processes, is to influence future events, level of noise cannot be too high. The transmitted influence on future events may involve the preservation of the efficiency of the ecosystem. Here is a point where both complementary aspects of the community focus, *viz.*, the structural and the trophic-dynamic.

Only with a certain degree of stability (that is, with a low level of noise) can animals put into effective use the information stored by the species, such as migrating where food really is or mates await. In turbulent water, it is impossible to obtain profit from a great part of such information as, for example, indirect responses and internal rhythms. In a highly stimulating paper, Quastler (1959) sets forth the basis for a quantitative study of the relations between the degree of integration of an ecological frame (the reduction of noise) and the possibility of a reduction in the number of bearings that an organism has to take in such environment (feature sampling). It is obvious that in a 'primitive' environment that introduces a high level of noise, species tend to have direct responses to different factors and find no use for a capacity of storing 'information' effective in the sampling of a few features; these species can maintain themselves by a high rate of multiplication, with high power output, but with a low efficiency. Thus, dunes, creeks, the limnetic and pelagic spaces can be considered as environments with a level of noise higher than forests, the depths of the sea, or the coral reefs.

Succession is a result of storage and utilization of information and the effect of noise

that reduces the amount of recoverable formation also defines the point where succession has to stop. Thus the maximum degree of maturity attainable by a community is controlled by environment.

It has been said previously that substitution of species, along succession, shifts information from the interindividual to the intraindividual level. The intraindividual channel involves the copy of genetic information and thermodynamically is cheaper or more efficient than the maintenance of structure by a feedback system such as is operating between species. A high content of information has to be coupled with a restricted feature sampling, only possible when the environment has a low noise level.

Migration and turbulence

Applications of this model are easy to grasp in connection with plankton communities, notwithstanding their general usefulness for all sort of communities. The transmission of information can also be used to analyze movements of planktonic organisms and also the part played by migration and turbulence in their movements.

Ashby (1957) offers an excellent starting point. With a large number of animals distributed in an environment, every individual in the place a at time t_1 has definite probabilities to be encountered in the places a,b,c . . . at time t_2. It is possible to write a matrix with the transition probabilities, and to compute the average state distribution and also the entropy or noise. The very fact that we have to substitute a deterministic model (such as including a statement that individual x will be met two hours later precisely at level b) by a system of probabilities, means that we have to contend with an amount of noise.

The information that fills the whole capacity of channel at the beginning (I_1) splits in two parts: information at the output (E) and noise (N). Perhaps the ratio E/I may be a good measure of what Bray calls "order or organization" and Quastler "integration." Quastler bases his measure on the ratio between the number of effective configurations and the number of possible ones.

If individuals remain fixed or change in a completely determinate way, the quotient $E/I = 1$. When movement is at random, the recovered information amounts only to the statement of number of species and number of individuals, and the quotient E/I is, of course, much lower. Such a quotient may be related to: 1) properties of the community, such as the swimming power of animals and the connections between organisms due to colony formation, epibiosis, etc., and 2) the turbulence of water. Now the problem is to put this into practical use.

Let us consider real populations subjected to births and deaths. It may be safely stated that when multiplication rate is low, average life long and fluctuations buffered, then the future situations are more predictable. Communities with such characteristics afford a channel with a lower noise level and may be considered as more organized.

With time, channel capacity may become larger, so that

$$I_1 < I_2 = E + N = I_1 + N'$$

and N' may be spoken of as a creative or "negative" noise.

Structural problems at the levels of specific and biochemical composition

We come again to our problem of bridging in some quantitative way the two aspects of a biotic community. Matter and energy flow can be expressed quantitatively in an obvious way. Information theory seems to offer a possibility to express quantitatively structural properties, at least such as are manifested at the level of assemblages of individuals. The translation of an energy budget into an information budget by simply converting calories into bits (Patten 1959) may give an idea of the amount of information to be expected in the whole organization of the community, but, for the moment, takes us no further.

When a mixed phytoplanktonic population is subjected to a sudden increase of the supporting capacity of the environment, the different species are led to take full advantage of their respectives rates of increase. As such rates differ widely, the result is a decrease of the diversity (I, in (1)

above); and one or a few species manifest strong dominance. If ecological succession proceeds, diversity rises again to attain a stable value or drops slightly at the end.

We can consider the same population also in another way, either as a complex of photosynthetic mechanisms, or more simply, as an assemblage of different assimilatory pigments. An animal population could be simplified also to the form of a mixture of selected easily analyzable, chemical compounds. Now, the interesting fact, manifested in natural populations of marine plankton as well as in experimental populations artificially assembled in culture vessels, is that changes of the "diversity" in the specific level and in the biochemical level show a striking parallelism or analogy. In the case of a sudden increase of nutrients, chlorophyll *a* increases at a much higher rate than the other pigments present in the plankton. Thus if we have calculated a sort of diversity index based upon the kinds of molecules of different pigments (chlorophylls and carotenes), such diversity index drops, as does the diversity index based on distribution of individuals into species. More labile components and also those having a key position (as chlorophyll *a*) respond more quickly than stable components with a more complex biochemical dependence (carotenes). Other chlorophylls are in an intermediate position. The parallelism is maintained also along ecological succession up to a certain point. After the initial outburst with rapid unequal increase of the pigments, the differences of concentration between the different pigments diminishes with diversification of the photosynthetic mechanisms just as there is a completion and diversification of the niche structure of the community in succession.

These relations are also invaluable in the study of the spatial distribution of populations. For example, in the layers of contact and mixing between two water masses, two cases can be identified: 1) the two water masses supply complementary nutrients, then growth of the populations in the boundary receives a push, biotic diversity drops and pigment diversity also diminishes, 2) there is no complementary enrichment; populations simply mix, biotic diversity increases as a result of the mixture in the boundary, but pigment diversity does not change if both populations are in a similar physiological state.

Relations between structure and energy flow

We need to find a model to visualize the relations between the structural changes, the flow of energy and the biomass. Biomass in one aspect can be imagined as mass of different species, in others as mass of different biochemical subsystems cutting or overlapping across the limits of species.

The energy flow is distributed in any case through a number of subsystems (species, photosynthetic mechanisms). Subsystems, which convert energy more efficiently into information, accumulate biomass at a higher rate.

Noise, in the form of fluctuations with time, a sort of turbulence, may restrict possible assemblages of species to those that are able to follow the fluctuations and that work with a low efficiency.

I feel unable to draw a quantitative expression of the way variations in the possible energy flow are reflected in the relative biomass supported. No doubt someone will provide the ecologists with a coherent and workable model. With it, dynamics of planktonic populations including the related problems of migration, stratification, influence of water turbulence, and the practical problems of analysis of phytoplankton populations through cell counts or through pigment analysis will enter in a new phase of scientific study.

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A COMPARATIVE STUDY OF IRRADIANCE, BEAM TRANSMITTANCE AND SCATTERING IN THE SEA NEAR BERMUDA[1]

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ABSTRACT

Comparative measurements of a number of optical properties of the sea were made at 6 stations and at depths down to 245 m near Bermuda. *In situ* determinations included attenuation of natural radiant energy and reflectance between 375 and 651 mμ, beam transmittance and scattering, as well as polarization 90° to the beam. In the laboratory, scattering and degree of polarization were determined at 90° from water samples, while scattering was also measured at 45° to the beam. Both photovoltaic cells and photomultipliers were used as required.

The irradiance and beam transmittance data indicate that the wide differences in attenuation at different stations are primarily due to variations in the amount of suspended matter both offshore and inshore provided that the coastal or partly enclosed water is in open communication with the sea. However, in Harrington Sound, which has only restricted communication with open water, the optical properties differ in indicating greater concentrations of yellow substance than at any other station.

Scattering coefficients measured at 45° and 90° in the laboratory show an approximately linear correlation for Castle Harbour and North Shore stations. In general the scattering coefficients at 90° are systematically correlated with the degree of polarization so that plotting p-log β yields a linear function whose slope depends on the nature of the particles suspended in the different water masses.

In situ measurements of scattering and polarization usually were different from those in the laboratory. In clear Sargasso Sea water, for instance, *in situ* polarization was greater (75–80%) than it was in the laboratory (60%) although the scattering coefficients in the sea were definitely smaller. Further work is required to account for such discrepancies. Comparisons of *in situ* polarization of natural radiant energy with polarization from a light beam show that natural polarization decreases more rapidly than that from an artificial light beam as the amount of scattering increases. This effect results from the dependence of submarine polarization not only on scattering but also on directionality of the underwater radiant energy which diminishes for daylight with the amount of suspended matter.

INTRODUCTION

As a continuation of the authors' research programs in optical and biological oceanography, a collaborative study of certain optical properties of sea water near Bermuda has been made both *in situ* and in the laboratory. The attenuation of radiant energy by absorption and by scattering was the main phenomenon studied. *In situ* determinations were made of natural underwater radiant energy, as well as the transmittance, scattering and polarization of light by sea water at 6 stations covering a wide range of optical conditions. Also the scattering and polarizing properties of a considerable series of water samples from these same stations were measured with photomultiplier scattering meters.

The results are relevant mainly to three different but interrelated problems: 1) analysis of the nature, size and distribution of scattering particles in the sea; 2) correlation of the polarization of submarine radiant energy with the optical properties of the types of water concerned; and 3) the further development of an hydography in which appropriate optical properties may be employed to characterize particular water masses; such properties may also be correlated with currents, winds or thermo-

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[5] Contribution No. 284.

clines. Because of the great importance of light in the sea to organisms living in it, these matters are of interest not only to physical oceanographers but also to biologists.

For example, the behavior of aquatic organisms in the photic zone, and more particularly their orientation, are affected by the angular distribution of underwater radiance. In addition polarized light patterns arising from light scattering in the water may be important here because specific sensitivity to this particular type of radiant energy is widespread in arthropods (Waterman 1960; Jander and Waterman 1960).

Five of the stations occupied were the same as those studied earlier for underwater light polarization by Ivanoff and Waterman (1958b, Fig. 5). They are numbered from 1 to 5 in a series with increasing turbidity ranging from the first in clear deep Sargasso Sea water to last in highly turbid shallow inshore water of Castle Harbour. Some data were also taken at a sixth station, 1b, which is located just south of Castle Roads and thereby has features combining those of inshore and oceanic waters.

ATTENUATION OF NATURAL RADIANT ENERGY BY SEA WATER

Radiant energy penetration

This function is relevant to a number of oceanographic problems which are not yet adequately solved. Over-all energy exchange and organic productivity may be mentioned in addition to some of the optical and biological questions cited above. In order to study the correlation of spectral transmittance with depth and water type, radiometric measurements were made down to 75 m where depth permitted. The wavelength range sampled extended from the near ultraviolet to the red. Mainly downward irradiance was recorded, but a few data taken on upward irradiance permit calculation of the water's reflectance (R = upward irradiance/downward irradiance).

Methods. For most of the measurements here reported, a simple irradiance meter provided with a photovoltaic cell was used.

Table 1. *Filter characteristics*

Color filter	λ_{max} mμ Nominal	With photomultiplier	With photovoltaic cell	Bandwidth at half max height mμ
G.A.B. interference filters	375	373	–	18
	430	429	428	16
	484	–	481	12
	514	513	511	12
	579	577	575	14
	625	623	621	13
	651	649	646	15
Schott filters				
2 mm UG1 + BG12	–	–	375	30
2 mm BG12 + CG5	–	–	465	40

This has adequate sensitivity for inshore shallow measurements, but some observations at oceanic Station 1 were carried out with a photomultiplier type of instrument previously described (Jerlov and Piccard 1959).

To obtain appropriate spectral band widths a series of filters was used. At the short wavelengths this presented no problems since reasonably narrow band width heterochromatic filters with adequate transmittance are available in the UV and the blue (Table 2). However, at longer wavelengths, interference filters are the only narrow band elements available. But, as they are usually used with small angles of incidence, their energy transmittance is too low for present purposes.

Nevertheless they can be adapted for use with a photovoltaic cell if their angular field is increased to get more flux into the cell. This does not affect either the band width or λ_{max} greatly, provided the angle of incidence does not exceed 15° (M. Fukuda 1958, unpublished data). Consequently a special irradiance meter was constructed by mounting the photovoltaic cell (37 mm diameter) at the considerable distance of 80 mm from the opal glass just inside the window (8 mm diameter) of the instrument. Seven G.A.B. interference filters with λ_{max}'s well spaced between 374 mμ and 651 mμ were placed between the window and the opal glass for particular measurements (Table 1). Unfortunately Filter 579 was damaged during the measurements so that

TABLE 2.—*Irradiance measurements with photovoltaic cell and Schott filters*

Station	Date	Depth (m)	Wavelength (mμ)			
			375		465	
			Percentage of surface light	Transmittance per m depth	Percentage of surface light	Transmittance per m depth
1	24/7/58	0	100	95.6	100	97.9
		20	41	96.2	65	97.0
		30	28	94.5	48	96.6
		40	16	93.7	34	96.2
		50	6.8	—	23	95.8
		60	—	—	15	94.0
		70	—	—	8.0	—
	7/8/58	0	—	—	100	97.5
		10	—	—	78	98.1
		25	—	—	63	97.0
		50	—	—	31	94.9
		75	—	—	8.5	—
1b	23/7/58	0	100	88.5	—	—
		5	54	90.1	—	—
		10	32	95.7	—	—
		20	14	94.9	—	—
		30	7.3	94.8	—	—
		40	3.9	94.7	—	—
		50	2.1	—	—	—
4	22/7/58	0	100	77.5	—	—
		2	60	80.0	—	—
		5	31	78.0	—	—
		10	9.0	76.0	—	—
		15	2.3	—	—	—
5	5/7/58	0	100	78.0	100	88.5
		2	61	81.5	78	93.1
		5	33	75.5	63	85.5
		10	8.1	—	29	—
	23/8/58	0	100	74.0	100	90.0
		2	55	70.0	81	82.5
		5	19	71.5	45	78.0
		10	3.5	—	13	—

observations in the yellow are lacking at Stations 3 and 4.

In the photomultiplier instrument the narrow band interference filters were the only ones employed. The λ_{max}'s actually present in the various filter-meter combinations used are listed in Table 1. Despite the several irradiance meters and filters utilized there are no serious discrepancies in the measurements, and agreement between the various sets of determinations may be considered satisfactory.

Data were collected from a launch for the inshore stations and from "R.S. Panulirus" at Station 1. In all cases the sun's zenith distance was between 20 and 30°.

Results. Exploratory observations were made first in the UV and the blue (Table 2). Among other things, the results for Station 1, 24/7/58, show a decreasing slope with depth when plotted on a semilogarithmic graph (Fig. 1). Then attention was concentrated on the over-all spectral distributions of radiant energy at Stations 1, 3, 4 and 5. Probably all data taken at Station 1 between 0 and 25 m may be considered together because the optical properties of this clear water are quite uniform from day

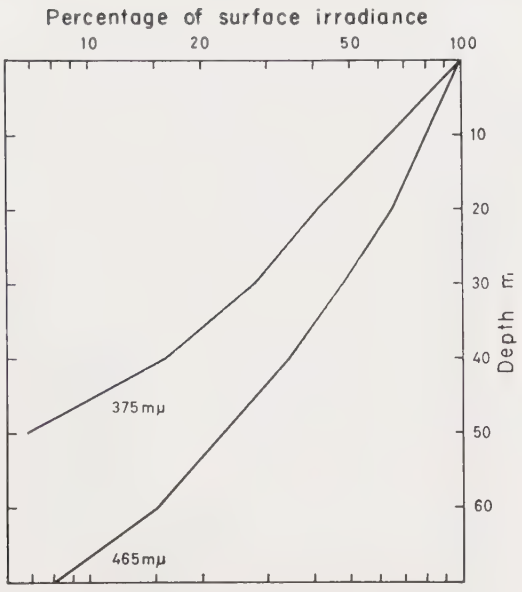

FIG. 1. Percentage of surface irradiance at different depths for blue (465 mμ) and for near ultraviolet (375 mμ) at Station 1.

to day. Calculated transmittances per meter depth are shown in Table 3 as well as in Figures 2 and 3. Maximum transmittances measured occur at 465 mμ (97.9%) for Station 1, at 481 mμ (89.5%) for Station 3, 465 mμ (85.5%) for Station 4 and 481 mμ (86%) at Station 5. However, if smooth curves are fitted to the data by eye, the maximum penetration in turbid water appears somewhat nearer 500 mμ than 475 mμ which has the greatest transmittance in the clearest water.

In the comparison between Stations 1 and 3 (Fig. 2), the difference between the spectral transmittance curves is greatest at the UV end and decreases steadily towards the red. Although the curves for Stations 4 and 5 (Fig. 3) are closely similar, there seem to be significantly greater transmittances for Station 4 in the red and for Station 5 at the short wavelengths.

Finally the data illustrate the variations in reflectance, R, with depth and water type (Table 4). At Station 1, R decreases toward greater depths while it shows a marked increase at Station 5. The values at shallow Stations 3 and 5 are caused in part by intense scattering by the abundant suspended particles and also by bottom reflection since R increases near the latter. At Station 1, R is small at the longer wavelengths and maximal at 450 mμ.

Discussion. The present Bermuda data may be given some perspective if they are compared with previous measurements from elsewhere. To begin with, the bending of the short wavelength curves at Station 1 is generally typical for light penetration in clear water (Jerlov 1951). Some interesting points emerge from the spectral transmittance curves if they are compared with the various water types in the Jerlov classification. Thus the upper and lower curves in Figure 2 represent Type I and Type III oceanic water respectively. In general the correspondence of the data at Stations 1 and 3 is good. However, the present observations at Station 1, except for one point, fall somewhat below the Type I curve; this agrees with Clarke's (1941) evidence that Bermuda offshore water is not as clear as the center of the Sargasso Sea.

Some discrepancies appear at the ends of the spectrum between the Station 3 data and the Type III curve, and these may suggest some fundamental differences between

TABLE 3. *Radiant energy transmittance per meter depth (%)*

Station	Date	Depth interval (m)	Wavelength (mμ)											
			373	375	428	465	481	511	513	575	577	621	646	649
1	24/7/58	0–20	–	95.6	–	97.9	–	–	–	–	–	–	–	–
1	7/8/58	0–25	96.2	–	–	97.8	–	–	95.9*	–	90.9*	–	–	72*
1	13/8/58	0–20	–	95.9	95.4	97.6	97.0	96.2	–	–	–	74	68	–
3	14/8/58	0–15	–	83.5	86.5	88.5	89.5	88	–	–	–	71.5	67	–
4	12/8/58	0–15	–	73	81	85.5	83.5	85	–	–	–	71.5	66	–
5	9/8/58	0–10	–	75.5	81	85	86	84.5	–	80	–	69.5	60	–

* These data obtained with photomultiplier; all other determinations with photovoltaic cell.

TABLE 4. *Reflectance* R *(%)*

Station	Date	Depth (m)	Wavelength (mμ)						
			375	429	465	513	577	623	649
1	7/8/58	0	3.3†	4.1*	4.1	2.6*	0.52*	0.22*	0.04*
		10	–	–	4.3	–	–	–	–
		25	–	–	3.4	–	–	–	–
		50	–	–	2.4	–	–	–	–
		75	–	–	2.1	–	–	–	–
	13/8/58	0	–	–	4.2	–	–	–	–
3	14/8/58	0	10	–	16	–	–	–	–
4	12/8/58	0	1.0	–	3.5	–	–	–	–
5	9/8/58	0	–	–	12	–	–	–	–
	23/8/58	0	8	–	11	–	–	–	–
		2	10	–	13	–	–	–	–
		5	16	–	17	–	–	–	–
		10	19	–	29	–	–	–	–

† This point added 23/8/58.
* These data obtained with photomultiplier; remainder with photovoltaic cell.

the water types studied. Thus beam transmittance meter measurements presented below indicate that Station 3 water contains no more yellow substance than offshore water. Hence, attenuation in the former must be due mainly to particles. On the other hand, the difference in transmittance between Type I and Type III oceanic water is associated with greater amount of yellow substance.

There is no classified type of oceanic water with as low transmittances as Stations 4 and 5. However, comparison of these two stations suggests that the differences in spectral transmittance (Fig. 3) arise from higher concentrations of yellow sub-

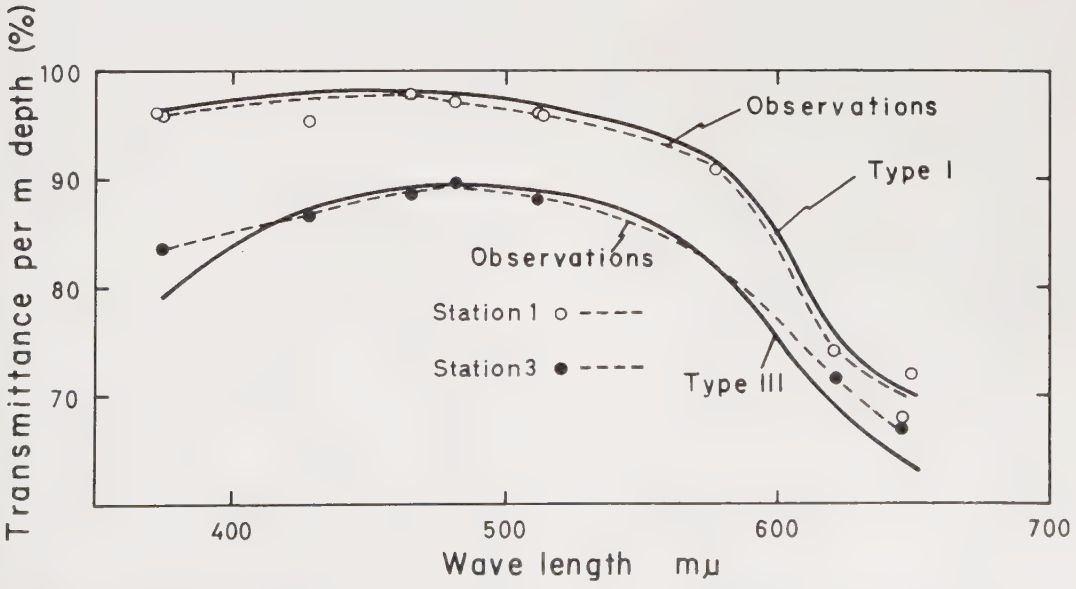

FIG. 2. Spectral transmittance curves for Stations 1 and 3 compared to those for Type I and Type III oceanic water respectively.

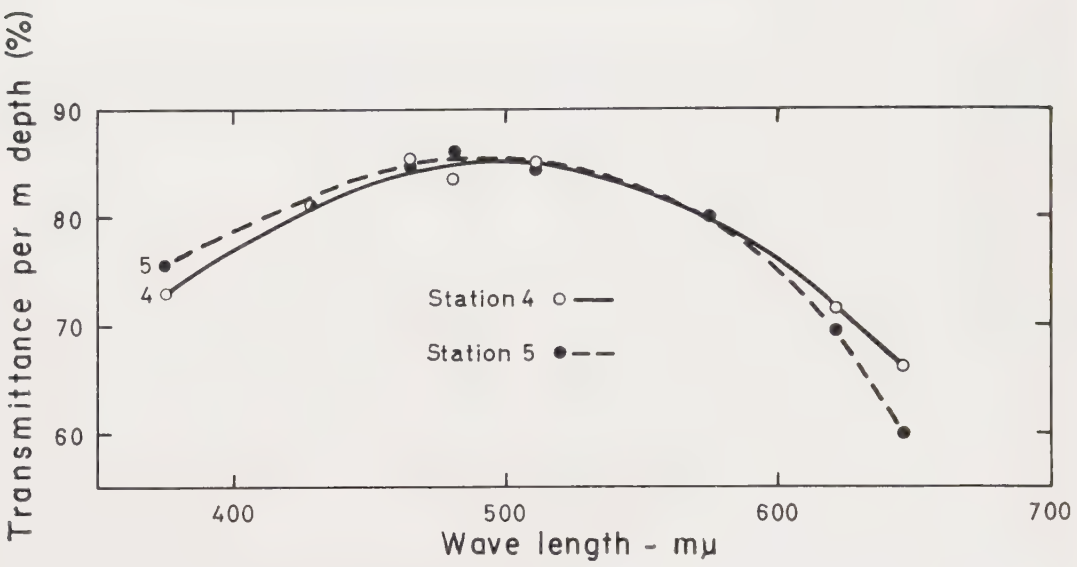

FIG. 3. Spectral transmittance curves for Stations 4 and 5.

stance at Station 4 because the greater particle abundance at Station 5 shows that the differences cannot be due to selective particle absorption. Although Station 5 is shallow, bottom reflections are not believed to have affected the results appreciably.

The upward irradiance data support the conclusions on water clarity from the daylight penetration measurements at Station 1. In the clearest water, maximum R values between 5 and 7% for small solar zenith distances occur at about 425 mμ (Jerlov 1951). The difference between this and the maximum transmittance is attributed chiefly to selective scattering (cf. Kalle 1939; Ivanoff 1953). But such selective scattering is significant only in the clearest waters and is evidently not as prominent at Station 1 where maximum R is near 450 mμ which is closer to maximum transmittance.

The light scattered from below is important in establishing the color of the sea. At Stations 3 and 5 suspended white mineral particles give the water a strong whitish appearance. A quite different situation occurs at Station 4. There R is quite low and much lower in the UV than in the blue which indicates the presence of yellow substance. Accordingly the blue produced by selective scattering is extinguished and replaced by a greenish blue color.

Beam transmittance

While important natural properties of water masses are related to their transmittance of radiant energy from sun and sky, certain other analytically useful measurements may be made using an artificial light source transmitted through a standard underwater light path. The attenuation so measured is due to absorption as well as scattering. Thus $c = a + b$ where c is the attenuation coefficient, a is the absorption coefficient and b is the total scattering coefficient. Transmittance (T) is defined as follows:

$$T = e^{-c}$$

Methods. The transmittance meter used is a simple type consisting of two cases, one containing a lamp and a lens, the other a photovoltaic cell. From the lamp case a collimated beam of light passes through one meter of water before it enters the cell case. The whole instrument is suspended from a conductor cable and is easily handled manually from a small boat.

Routinely, the meter was used with the following color filters attached outside the window of the photocell case: Schott RG

1 (λ_{max} 655 mμ, band width to 0.5 transmittance 50 mμ) and UG 1 + BG 12 (λ_{max} 380 mμ, band width 30 mμ).

Meter calibration is important in utilizing such an instrument to study absorption and scattering in sea water. The present transmittance meter was standardized by comparison with spectrophotometers whose characteristics were known. The transmittance is proportional to the ratio u_w / u_a, where u_w is the reading on the microammeter when the meter is in water, and u_a the reading when the meter is in air, the latter being used as a standard (Jerlov 1944). From the standardization it is found that the transmittance per meter ($T\%$) and the attenuation coefficient (c) are related to this ratio as follows:

$$T_{380\ m\mu} = 53\, u_w / u_a$$

$$T_{655\ m\mu} = 59\, u_w / u_a$$

The meter was not shielded from daylight. Consequently spuriously high readings could be obtained during the day unless such interferring light was subtracted from the readings. In the red the resulting correction was trivial, but in the UV it was quite high near the surface. Nevertheless, accurate corrections could be made because of the stable daylight conditions at the time of measurement. In the data here reported attention is directed to the attenuation caused by particles and dissolved substances by eliminating the attenuation due to the water itself, c_w. According to Clarke and James (1939) these values are:

$$c_w = 0.04 \text{ per m at } 380\ m\mu$$

$$c_w = 0.30 \text{ per m at } 655\ m\mu$$

Initially the UV transmittance patterns of Bermuda waters were studied on 3/7/58 in a series of stations starting from Castle Harbour (Station 5) southward to Station 2 in Castle Roads to Station 1b offshore. Later (5/7/58) further data were taken at Station 5 and neighboring Stations 5a and 5b. Finally comparative data were taken at Stations 1, 1b, 3, 4 and 5 in the red as well as the UV. The measurements at Stations 1 and 1b extended down to 50 m and close to the bottom at the others.

Results. Transmittance per meter is summarized in Table 5 and, for Stations 1b, 2

TABLE 5. *Beam transmittances (T) and attenuation coefficients (c)*

Station	Date	Depth (m)	Wavelength (mμ) 380 $T\%$	380 $c^* - c_w$	655 $T\%$	655 $c - c_w$
1 Sargasso Sea	24/7/58	0	75	0.25	69	0.07
		10	78	.21	67	.10
		20	81	.17	67	.10
		30	82	.16	67	.10
		40	81	.17	67	.10
		50	78	.21	68	.08
1b Castle Roads	3/7/58	0	81	0.17	–	–
		2	72	.29	–	–
		5	72	.29	–	–
		10	75	.25	–	–
		15	72	.29	–	–
		20	69	.33	–	–
		30	72	.29	–	–
		40	72	.29	–	–
		50	75	.25	–	–
	23/7/58	0	64	0.41	60	0.21
		5	62	.44	60	.21
		10	70	.32	62	.18
		20	76	.23	64	.15
		30	75	.25	64	.15
		40	75	.25	64	.15
		50	78	.21	64	.15
2 Castle Roads	3/7/58	0	63	0.42	–	–
		2	63	.42	–	–
		5	60	.47	–	–
		10	63	.42	–	–
		15	65	.39	–	–
	23/7/58	0	–	–	60	0.21
		2	–	–	61	.19
		5	–	–	62	.18
		10	–	–	64	.15
		15	–	–	66	.12
		17	–	–	63	.16
3 North Shore	16/7/58	0	63	0.43	59	0.23
		2	66	.40	60	.21
		5	66	.40	60	.21
		10	63	.43	56	.28
		12.5	63	.43	56	.28
		15	37	.96	41	.59
		16	–	–	27	1.00
	21/7/58	0	38	0.93	–	–
		2	41	.85	–	–
		5	41	.85	–	–
		10	38	.93	–	–
		12.5	31	1.14	–	–
		15	28	1.23	–	–

TABLE 5 (*Continued*)

Station	Date	Depth (m)	Wavelength (mμ) 380 $T\%$	380 $c^{\circ}-c_w$	655 $T\%$	655 $c-c_w$
4 Harrington Sound	22/7/58	0	55	0.56	55	0.30
		2	55	.56	55	.30
		5	50	.66	55	.30
		10	53	.60	53	.33
		15	50	.66	55	.30
		16	44	.78	55	.30
		17	43	.81	52	.35
		19	40	.87	49	.41
		19.7	–	–	40	–
5 Castle Harbour	3/7/58	0	53	0.59	–	–
		2	53	.59	–	–
		5	37	.96	–	–
		10	24	1.39	–	–
	5/7/58	0	50	0.65	50	0.37
		2	52	.61	49	.41
		5	46	.69	49	.41
		10	26	1.31	32	.84
	17/7/58	0	42	0.82	–	–
		2	42	.83	–	–
		5	41	.84	–	–
		10	28	1.23	–	–
		11	19	1.62	–	–
		12	16	1.79	–	–
	18/7/58	0	34	1.04	39	0.64
		2	31	1.13	40	.62
		5	31	1.13	40	.62
		7.5	27	1.27	31	.87
		10	25	1.35	31	.87
		12	14	2.00	11	1.91
5a Castle Harbour	3/7/58	0	43	0.80	–	–
		2	42	.83	–	–
		5	31	.98	–	–
		10	14	1.93	–	–
	5/7/58	0	–	–	43	0.54
		2	–	–	42	0.57
		5	–	–	41	0.59
		10	–	–	24	1.12
		11	–	–	18	1.42
5b Castle Harbour	5/7/58	0	–	–	41	0.59
		2	–	–	44	0.52
		5	–	–	44	0.52
		8	–	–	29	0.94
		10	–	–	25	1.08
		11	–	–	23	1.17

° Unit: m^{-1}

and 5, plotted as a function of depth in Figure 4. Station 5 in Castle Harbour shows low surface transmittance as well as a rapid decrease from 2 m toward the bottom. At

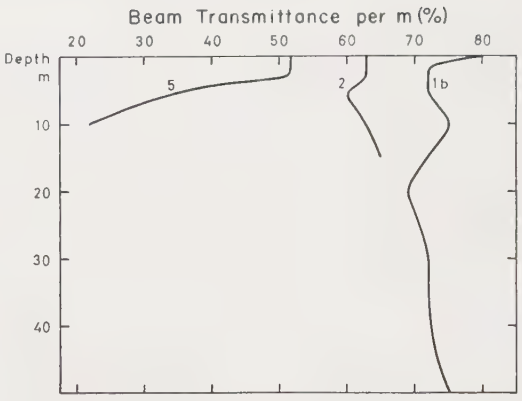

FIG. 4. Beam transmittance per meter for ultraviolet (380 mμ) at Stations 1b, 2 and 5.

Station 2 more homogeneous and less turbid water is indicated, and at Station 1b the observed transmittance approaches that in offshore water. Stations 5a and 5b, which are two locations in Castle Harbour near Station 5 itself, indicate that the curve for Station 5 in Figure 4 is typical for Castle Harbour which showed little horizontal variation although tidal currents and their direction of flow have a strong influence on the distribution of water masses in the region of transition to the open sea. In Castle Harbour itself the homogeneity of the underwater optics demonstrates the thorough mixing effect of tidal currents.

Wavelength sensitivity of the transmittance can be epitomized by plotting attenuation coefficients ($c-c_w$) for the red against those for the UV (Fig. 5). For Stations 1, 1b and 3 mean coefficients have

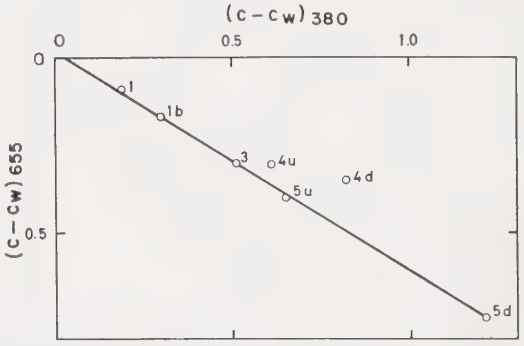

FIG. 5. Relationship between beam attenuation coefficients for red (655 mμ) and ultraviolet (380 mμ).

been used to represent the whole water column. For Station 4, strata above the thermocline, which started at 12 m, are shown as 4u and those below this depth as 4d. For Station 5 the range of attenuation coefficients is so large that those above 1.00 are averaged at one point 5d and those below at 5u.

Discussion. Evidently, the high attenuation coefficients found in Castle Harbour are the result of particulate matter. Large amounts of calcium carbonate particles, originally derived from the rocks, are lifted by tidal currents and to some extent by turbulence caused by the wind. An influence of bottom currents on particle distribution has been demonstrated by Joseph (1954) who showed that in the North Sea a rhythmic rising and sinking of sediment takes place mainly through the agency of tidal currents. Similar fluctuations occur in the Adriatic Sea (Jerlov 1958). If the currents transporting the particles are horizontal, the particle distribution should be logarithmic with depth. Suggestions of such a distribution have been found in Castle Harbour, but because of the shallow water, this phenomenon is not likely to be clearly developed.

The essentially linear relation shown between attenuation coefficients in the UV and in the red, except at Station 4 (Fig. 5), may be explained as follows. As first shown by Joseph (1949), such comparative measurements at the ends of the spectrum can be used to demonstrate the selective absorption due to yellow substance and to particulate matter. These may be distinguished by the following formula for the absorption coefficient due to yellow substance at 480 mμ which was derived by Jerlov (1955).

$$a_y = (c - c_w)_{380} - K(c - c_w)_{655}$$

Here K is a factor dependent on the optical properties of an average particle. Effective application of this relation has been made in the Baltic where large areas were found to be characterized by $K = 2.0$.

Figure 5 indicates that the above equation holds for the attenuation coefficients at Bermuda Stations 1, 1b, 3 and 5 whose points lie close to a straight line with a K of 1.6. The corresponding absorption coefficient for yellow substance is $a_y = 0.03$. This is a plausible value, but the actual figure obtained will be highly dependent on standardization factors. These relations indicate that inshore Bermuda waters, being essentially in free exchange with the ocean, constitute basically the same water mass varying in its amount of particulate materials.

However, Station 4 in Harrington Sound, which has poor communication with the ocean, shows optically distinctive features. The thermocline starting at 12 m suggests stagnant bottom water in which production of yellow substance from sinking organic matter is likely to occur. This is consistent with the observed high UV absorption for the deeper layers (point 4d, Fig. 5); also the position of point 4u (Fig. 5) suggests that even the upper layers of Harrington Sound contain more yellow substance than the other stations sampled.

To test the alternative hypothesis that the high UV absorption at Station 4 was due to particle characteristics, the relation between $(c - c_w)$ for the UV and the red may be compared with particle scattering at 45° to the beam, measured on water samples from the corresponding stations (Figs. 6 and 7). All available data are used, but unfortunately $(c - c_w)_{655}$ is missing for Station 3. In general the relation between attenuation coefficients and scattering is linear although there are some deviations. Thus in the red, Station 4 is close to the straight line through the other stations (Fig. 7), but in the UV the point for Station 4 is considerably below the line, which supports the evidence for higher yellow substance in Figure 5. The fact that the points for Station 1 in both Figures 6 and 7 are above the line may be due to a smaller average particle size which might be expected in offshore water and which would be apt to result in stronger scattering.

Conclusions. All the optical data obtained both from irradiance measurements and from beam transmittance measurements indicate that at the various stations studied, except in Harrington Sound, the inshore waters differ from those offshore mainly in the amount of suspended particulate mate-

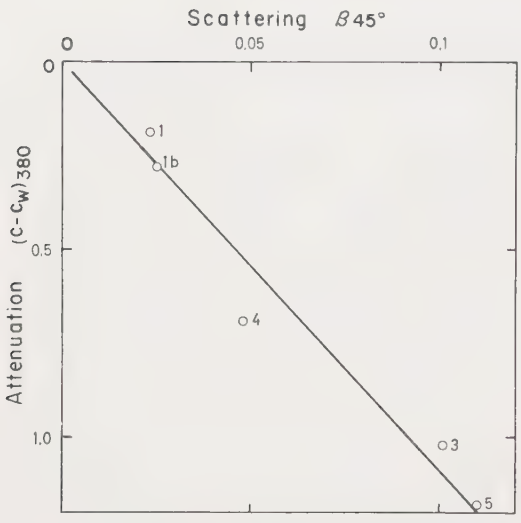

FIG. 6. Beam attenuation coefficient for ultraviolet $(c - c_w)_{380}$, as a function of 45° scattering, β (45°).

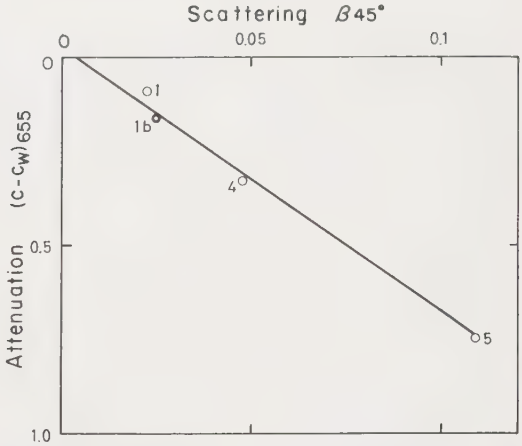

FIG. 7. Beam attenuation coefficient for the red $(c - c_w)_{655}$, as a function of 45° scattering, β (45°).

rial. All evidence similarly points to the presence of yellow substance in Harrington Sound at concentrations greater than any of the other stations.

SCATTERING AND POLARIZATION OF RADIANT ENERGY BY SEA WATER

Measurements on water samples

The radiant energy scattering properties of sea water appear to provide the most sensitive physical indication of the quantity and nature of the particles in suspension. For a given substance the scattering coefficient is proportional to the total surface of the suspended particles if their dimensions are greater than 2 μ (Jerlov 1955, Fig. 1). For different substances, the proportionality constant in this relation varies markedly (Jerlov 1955, Table 4). The study of particle distribution by means of scattering measurements has permitted various water masses to be distinguished (Jerlov 1953, 1958, 1959; Ivanoff 1959c).

In addition the polarization of scattered radiant energy[6] provides information complementary to that found directly from measurements of scattering coefficients. Since depth, direction of line of sight, cloud cover and other factors influence polarization measurements of radiant energy from sun and sky, the polarizing effect of suspended particles themselves may best be studied in artificial light (Ivanoff 1957, p.52). In such measurements in the laboratory radiant energy scattered at right angles to an approximately parallel beam directed through the sample is recorded radiometrically so that both the degree of polarization (p) and the scattering in a given direction (β) are obtained (Ivanoff 1958a, 1958b, 1959b).

As mentioned above, β is proportional to the total particle surface and largely independent of particle form or dimensions. But p mainly depends on the shape and size of the particles and only slightly on their total surface or number. Unfortunately, these relationships are relatively simple only when the particles all consist of one substance. In the sea complications are introduced by the various kinds of suspended particles which may be present as, for example, when comparing water rich in suspended mineral matter with water containing much phytoplankton. Nevertheless, determination of both p and β provided more insight into the suspended matter than scattering data alone.

Methods. Satisfactory techniques for

[6] Detailed references to this field are given in Waterman (1954) and Ivanoff and Waterman (1958a, 1958b).

TABLE 6. *Polarization and scattering (90°) by Station 1 water samples*

Depth (m)	11/9/58 (bottom: 950 m)		12/9/58 (bottom: 900 m)		Averages	
	p%	log β	p%	log β	p%	log β
5	55	0.69	59.5	0.54	57	0.61
10	–	–	–	–	–	–
20	–	–	59	0.48	–	–
40	52.5	0.74	50.5	0.67	51.5	0.70
60	48	0.83	59	0.72	53.5	0.77
80	55	0.78	61	0.62	58	0.70
100	–	–	61	0.56	–	–
110	–	–	–	–	58	0.57
120	55	0.70	–	0.45	–	–
140	57.5	0.76	60	0.43	59	0.60
160	61	0.52	60	0.49	60.5	0.51
180	66.5	0.39	63	0.28	65	0.34
200	64.5	0.58	60	0.34	62	0.45
200	64	0.52	–	–	–	–
220	–	0.50	62.5	0.43	–	0.46
240	61	0.51	61	0.46	61	0.48

measuring p and β in the laboratory depend on the solution of problems relating to two phases of the subject: 1) instrumental design and calibration, and 2) adequate collecting and storing of the water samples.

Two scattering meters were used. One of these, already described by Ivanoff (1958a), measures scattering at 90° to an incident beam of white light 1–2 cm in diameter. This instrument, which permits the determination of both p and β, has little internal scattered light but does require a large water sample (500 ml). In use the instrument was standardized relative to a glass cube for which β at 90° is taken as 10. Since this standard scatters at this angle about ten times as much light as extremely clear sea water, β is about 1 for the clearest waters. The optical measurements with this meter usually could not be made at sea and were carried out in the shore laboratory within three hours of collection time.

TABLE 7. *Polarization and scattering (90°) by Station 2 water samples (23/7/58)*

Depth (m)	Sample 1		Sample 2		Averages	
	p%	log β	p%	log β	p%	log β
0	59.5	0.77	60	0.65	60	0.71
5	59	0.69	60	0.71	59.5	0.70
10	59	0.65	61	0.53	60	0.59
15	60	0.59	61	0.48	60.5	0.54
17	50.5	0.78	56	0.60	53	0.69

The other scattering meter, already described by Jerlov (1958) measures scattering at 45° to an incident beam of white or blue light 1 cm in diameter. Only β was determined with this instrument. Samples are conveniently introduced into the light beam in small bottles (150 ml) so arranged that scattering from their walls was negligible at 45°. The measurements were made as soon as possible after the collection of water samples, usually within an hour. To do this at Station 1, the determinations were made at sea with the meter mounted in the laboratory of the research ship.

The water sampling procedure employed was as follows. Ekman bottles lined with paraffin to minimize contamination were used to collect water in the usual way. In most cases a second sample was collected less than one-half hour later at the same place to check the reproductibility of the whole method. Warming of the sample can

TABLE 8. *Polarization and scattering (90°) by Station 3 water samples*

Depth (m)	16/7/58				21/7/58				14/8/58				Averages	
	Sample 1		Sample 2		Sample 1		Sample 2		Sample 1		Sample 2			
	p%	log β	p%	log β	p%	log β	p%	log β	p%	log β	p%	log β	p%	log β
0	54.5	0.76	54	0.79	51.5	1.28	52.5	1.28	45	1.38	46.5	1.33	51	1.14
5	55	0.77	54	0.82	50.5	1.30	51	1.33	45	1.39	47	1.31	50.5	1.15
10	53	0.98	52.5	1.00	48	1.43	48.5	1.41	46	1.48	47	1.41	49	1.28
12	54	0.99	52.5	1.03	–	–	–	–	–	–	–	–	–	–
14	54	0.93	45	1.52	–	–	–	–	–	–	–	–	–	–
15	–	–	–	–	46.5	1.60	46.5	1.64	44	1.57	44	1.66	46	1.50
16	48	1.47	43	1.64	45	1.66	45	1.71	–	–	–	–	–	–

TABLE 9. *Polarization and scattering (90°) by Station 4 water samples*

Depth (m)	22/7/58				29/7/58				12/8/58				Averages	
	Sample 1		Sample 2		Sample 1		Sample 2		Sample 1		Sample 2			
	$p\%$	$\log \beta$	$p\%$	$\log \beta$	$p\%$	$\log \beta$	$p\%$	$\log \beta$	$p\%$	$\log \beta$	$p\%$	$\log \beta$	$p\%$	$\log \beta$
0	56	0.81	56	0.82	55	0.99	54	0.97	52	1.02	52.5	1.02	54	0.94
5	54.5	0.89	53	0.96	49	1.03	50.5	1.08	47.5	1.18	50.5	1.10	51	1.04
10	50.5	0.97	51.5	1.02	50.5	1.06	51.5	1.09	51.5	1.07	49	1.10	50.5	1.05
14	54	0.91	54	0.90	55	0.99	55	0.96	49	1.18	47	1.22	52	1.02
16	–	–	–	–	–	–	–	–	42	1.32	46	1.28	–	–
17	50	1.05	49	1.01	49	1.08	50.5	1.06	–	–	–	–	46.5	1.20
18	–	–	–	–	–	–	–	–	38	1.49	47	1.34	–	–
19	52.5	1.21	51.5	1.14	51.5	1.29	49	1.21	–	–	–	–	–	–

induce carbonate precipitation which may strongly influence both p and β[7].

Results. The values of p and β obtained at the various stations with the 90° scattering meter are presented in Tables 6–10. In all cases samples were collected on more than one day and in a majority two casts were made on the same day. At Stations 3, 4 and 5, where the water was shallow and rich in suspended mineral particles, p and β near the bottom could vary markedly from one cast to the next. In contrast, variations in $\log \beta$ away from the bottom are usually less than 0.05 from one cast to the following and only rarely reached 0.08. Variations in paired determinations of $p\%$ not too close to the bottom usually are not more than 1.5% and only reached 3.0% exceptionally.

At Stations 1 and 2 where the water was deep, clear and less rich in mineral particles, the results are much more variable since $\log \beta$ may vary by 0.2 or more. Variations in $p\%$ are usually less than 13% but are much greater in certain cases where $\log \beta$ is unusually high.

Variations in optical properties of the water may also be seen from day to day or week to week. At the shore stations such changes are great as would be expected from previous knowledge. Wind and tide are the main factors responsible for variations of this kind. Although their effects have not been systematically studied in the

[7] A drop of acid in the sample will dissolve this carbonate and rapidly diminish these parameters.

TABLE 10. *Polarization and scattering (90°) by Station 5 water samples*

Depth (m)	17/7/58				18/7/58				19/7/58			
	Sample 1		Sample 2		Sample 1		Sample 2		Sample 1		Sample 2	
	$p\%$	$\log \beta$	$p\%$	$\log \beta$	$p\%$	$\log \beta$	$p\%$	$\log \beta$	$p\%$	$\log \beta$	$p\%$	$\log \beta$
0	55	1.24	54	1.25	53	1.40	53	1.40	47.5	1.56	47	1.60
5	55	1.25	55	1.27	50	1.45	51.5	1.48	47	1.59	46	1.62
7.5	53	1.35	54	1.34	45	1.65	46	1.65	46.5	1.62	46	1.63
10	54	1.47	53	1.45	45	1.64	46.5	1.65	47	1.65	46	1.66
12	53	1.83	53	1.79	46.5	1.87	47.5	1.92	45	1.76	46	1.75

Depth (m)	9/8/58		14/8/58				Averages	
	Sample 1		Sample 1		Sample 2			
	$p\%$	$\log \beta$	$p\%$	$\log \beta$	$p\%$	$\log \beta$	$p\%$	$\log \beta$
0	49	1.38	48	1.53	47	1.54	50.5	1.43
5	47	1.49	47	1.51	45	1.55	49	1.47
7.5	46	1.49	47	1.53	44	1.58	47.5	1.54
10	45	1.82	45	1.88	45	1.83	47	1.67
12	35	2.37	–	–	–	–	46.5	1.90

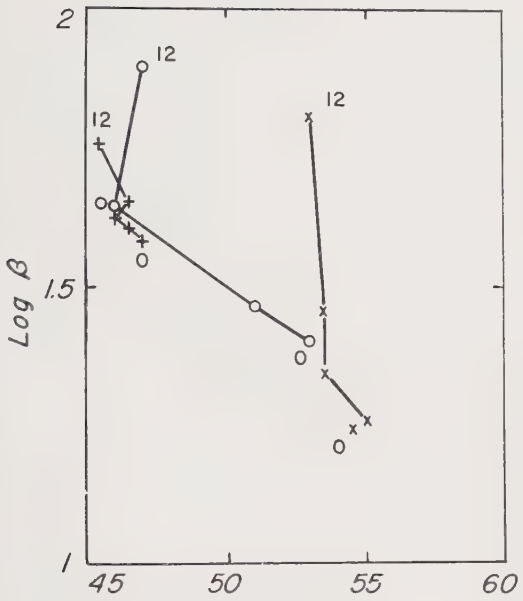

FIG. 8. Plots of $p\%$–$\log \beta$ obtained at Station 5 (17/7/58, x; 18/7/58, o; 19/7/58, +) at depths between 0 and 12 m. The intermediate samples were taken at 5, 7.5 and 10 m. Each diagram represents the average values for two successive casts of the water bottles (Table 10).

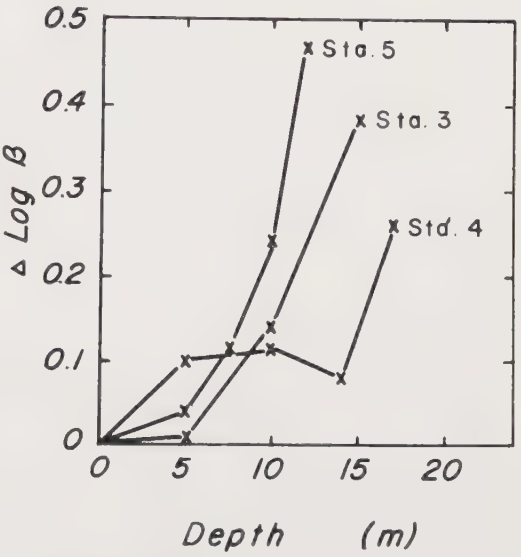

FIG. 9. Variations in $\log \beta$ as a function of depth for Stations 3, 4 and 5. Average values have been taken from Tables 8, 9 and 10 thus representing the average scattering properties of the water masses between about 15/7/58 and 15/8/58.

present measurements, some variations at Station 5 can be correlated with wind induced turbulence (Fig. 8). Thus on 17/1/58, after several calm days, $\log \beta$ ranged from 1.25 at the surface to 1.81 at 12 m which was 1–2 m above the bottom. Corresponding values of $p\%$ ranged from 55% to 53% indicating that particle size was essentially the same through the water column. On 18/7/58 after a night of strong wind, $\log \beta$ was greater at all depths whereas $p\%$ had become much smaller at depths greater than 5 m indicating that large particles stirred up from the bottom were still within 5 m of the surface. On 18/8/58, after two days of continuing strong wind, the mixing was more complete since the conditions previously limited below 5 m now extended right to the surface.

If the average data for $\log \beta$ at the shore stations are considered, the mean depth distribution of the scattering coefficient over a period of about one month may be examined (Fig. 9). At Stations 3 and 5 $\log \beta$ increases more and more rapidly as the bottom is approached; on the other hand the scattering coefficient at Station 4 passes through a secondary maximum at 10 m. This is undoubtedly correlated with the strong thermocline present at this Harrington Sound Station between 12 and 14 m (Fig. 10).

The results obtained with the 45° scattering meter are summarized in Table 11. These were all taken on samples from the first cast of the water bottles. The data for Stations 1b, 2, 3, 4 and 5 are quite consistent with those obtained at the same stations with the 90° meter. The Sargasso water (Station 1), measured at 45° only, shows a particle content slightly lower than that in Castle Roads (Station 1b).

Discussion. At the different stations as studied with the 90° scattering meter the sources of error and their magnitude varied. Thus at Stations 3, 4 and 5 the great variations in $\log \beta$ and $p\%$ found close to the bottom on successive casts were undoubtedly due to the sharp increase in turbidity towards the bottom. This would cause trivial differences in the depth of the Ekman bottle to have large effects on the re-

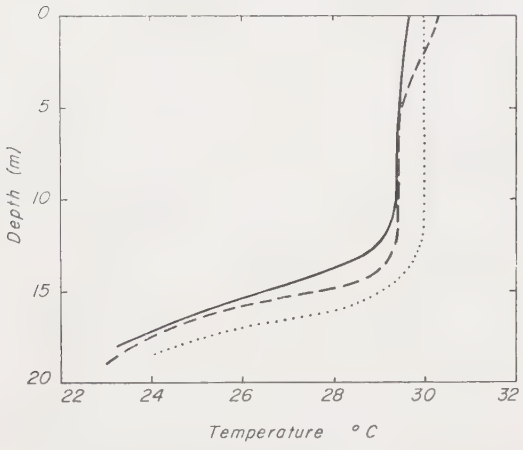

FIG. 10. Relation at Station 4 between water temperature and depth as recorded with a bathythermograph on 22/7/58 (continuous line), 29/7/58 (broken line) and 12/8/58 (dotted line).

sults obtained. On the other hand the considerable variations in $\log \beta$ at Station 1 are probably due to the large effects which slight sample contamination would have in extremely clear water.

Because β and p are important independent optical parameters of a water mass, the use of a p–β diagram has been proposed (Ivanoff 1958a) as a means of characterizing conditions at a particular station. When such a plot is made for a station at which

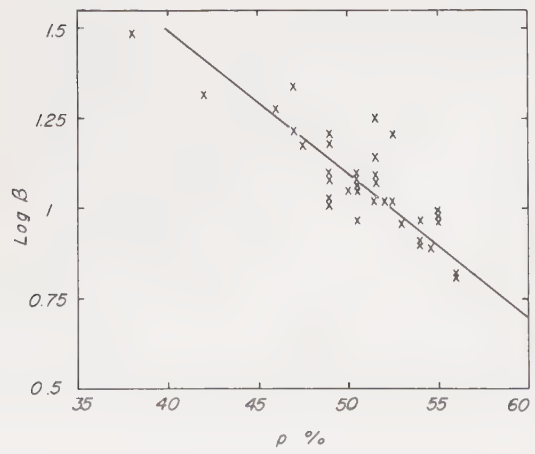

FIG. 11. In a p%–$\log \beta$ plot the data for Station 4 (Table 9) fall close to a straight line with a slope $\left(\frac{\Delta \log \beta}{\Delta p}\right)$.

TABLE 11. *Scattering (45°) by water samples*

Station	Date	Depth (m)	β (45°)
1	13/8/58	0	20
		10	28
		25	20
		50	30
1b	23/7/58	0	35
		10	25
		20	22
		30	24
		40	22
		50	19
2	23/7/58	0	28
		5	25
		10	25
		15	24
		17	25
3	21/7/58	0	59
		5	73
		10	94
		15	146
		16	132
4	22/7/58	0	30
		5	38
		10	52
		15	39
		17	53
		19	76
	17/7/58	0	58
		5	80
		8	80
		10	105
		12	195
5	18/7/58	0	79
		5	92
		7.5	124
		10	137
		12	275
	19/7/58	0	109
		5	138
		7.5	144
		10	152
		12	2,400

a considerable number of data are available, p and β fall roughly on a single curve suggesting their statistical interdependence; if p and $\log \beta$ are plotted, this curve approaches a straight line, which is one reason $\log \beta$ has been used above (Fig. 11). In general these relations are such that the smaller the scattering coefficient is, the larger the polarization coefficient. In other

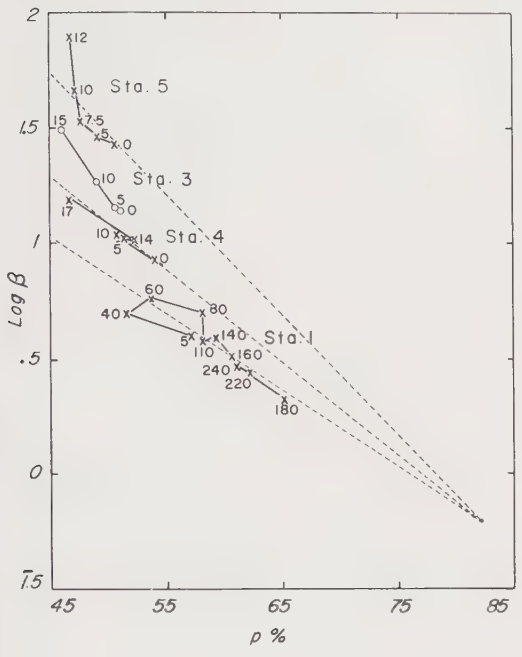

FIG. 12. Average p%–log β diagrams for Stations 1, 3, 4 and 5. For Stations 1, 4 and 5 the two regression lines for each may be combined into a single straight line passing through the point representing optically pure water ($p = 82\%$, $\log \beta = -0.2$). The slope of this straight line apparently depends on the nature of the particles suspended in the water.

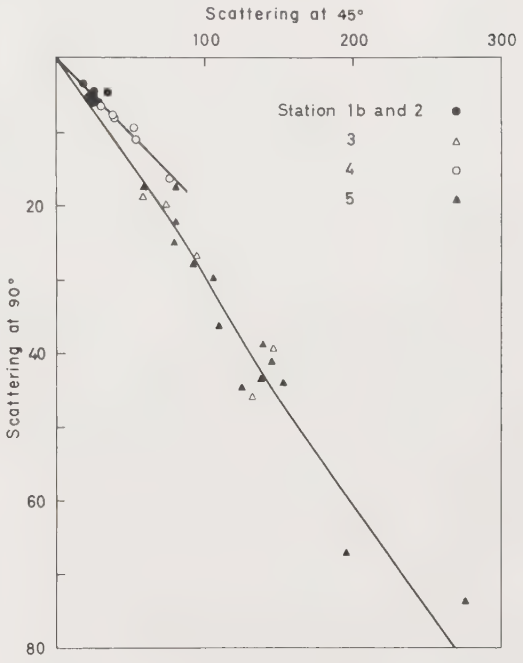

FIG. 13. Comparison of relative scattering at 45° and 90° to the beam.

words the more turbid the water, the greater the size of the suspended particles.

If $p - \log \beta$ diagrams are made for Stations 1, 3, 4 and 5, the slopes of the corresponding straight lines are different for each station (Fig. 12). Presumably this indicates the presence of different kinds of particles since for a given p, β varies with the optical properties of the particles and hence their nature. The effect of different kinds of particles on the $p - \log \beta$ diagram may be used to deduce the value for optically pure water which has no particles in suspension. Obviously the $p - \log \beta$ curves must intersect at a single point. If, as seems likely, these functions remain linear for extremely small amounts of suspended material, then their intersection should indicate the point typical of pure water.

To test this possibility rigorously the classic methods of statistical correlation need to be used. These involve the computation of the two regression lines which fit the data for each station. Since p for optically pure water is about 0.82 at 90° to the direction of transmission, the corresponding values of $\log \beta$ can be calculated from the regression line equations. Unfortunately, the 8 values of $\log \beta$ do not agree closely, particularly those for Station 3. However, if the average value of $\log \beta$, when $p = 0.82$, is taken as 0.2 for Stations 1, 4 and 5 and the two regression lines for each station are averaged, then three corresponding straight lines may be obtained running through the point for optically pure water (Fig. 12). When the average p–$\log \beta$ points are plotted, a rough correspondence is evident between the straight lines determined above and the observational data. The different slopes of the three straight lines would appear then to represent statistically the nature of the

particles suspended in the three water types.

A comparison of $\beta(45°)$ and $\beta(90°)$ is of interest, but as the two meters were not standardized against each other, only a relative comparison is possible. The available data from Stations 1b–5 have been employed to plot the corresponding values of $\beta(45°)$ and $\beta(90°)$ (Fig. 13). The points for Stations 3 and 5 are well fitted by a straight line while those for Station 4 may fall on another straight line. Such consistent results prove that sampling and measuring errors are not large and seem to vitiate the objection that scattering values would not represent the water sampled.

In situ *measurements*

Certain difficulties such as contamination, the settling of suspended particles in water samples and scattering and reflection of light within the laboratory instrument can be avoided if scattering and polarization measurements are made *in situ*. On the other hand, the presence of radiant energy from sun and sky and from bioluminescence (Clarke and Hubbard 1959) as well as the scattering of the test light beam by the surrounding water may have a deleterious effect on *in situ* measurements. A suitable shield might eliminate these side effects, but it in turn may introduce reflection of the test light and also reduce the water circulation.

Methods. The scattering and polarization meter used in these measurements (designed by Ivanoff 1958, unpublished) is exactly the same in principle as the 90° scattering meter used in laboratory measurement of water samples. The scattered light from a collimated test beam passes through a polarizing prism and then through a quarter-wave plate to eliminate possible artifacts arising from the differential sensitivity of the photomultiplier to various polarization planes. Relative measurements of the scattering coefficient are made using a glass cube as standard. Details of the design and construction of this instrument are shown in Figure 14.

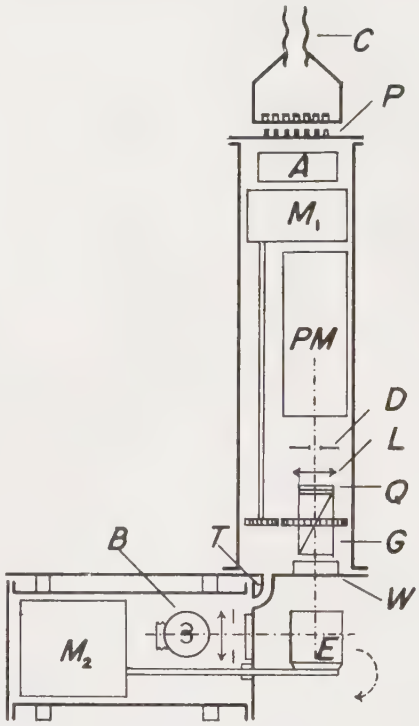

FIG. 14. Diagram of the remote control scattering meter-polarimeter. A, compartment containing activated alumina to keep the inside of the case dry; B, light source for incident beam; C, armored multiconductor cable (250 m long); D, diaphragm; E, glass calibrating cube, shown in position for standarization; G, polarizing prism; L, objective lens; M_1, motor to rotate the polarizing prism and quarter-wave plate; M_2, motor to move calibrating cube into and out of beam as shown by arrow drawn with broken line; P, plug for attaching cable; PM, photomultiplier; Q, quartz quarter-wave plate; T, tube to carry electrical connections into small pressure case; W, Plexiglas window with essentially zero photoelastic effect. The larger of the two pressure cases is about 70 cm high.

In use the 10-conductor cable for this instrument proved inadequate for its support; it had to be lowered on the ship's hydrographic cable. Another practical difficulty which appeared in use related to the speed of the polarizing prism's rotation. As designed, this was too fast for the time constants of the measuring system. Consequently accurate measurements of p could be made only with intermittent prism rotation although β could be determined adequately during rotation. Also the noise level

TABLE 12. In situ *polarization and scattering (90°) at Station 1*

Depth (m)	2/9/58 (bottom: 1250 m)		11/9/58 (bottom: 960 m)		12/9/58 (bottom: 2650 m) Sample 1		12/9/58 (bottom: 2650 m) Sample 2		12/9/58 (bottom: 875 m)		12/9/58 Averages	
	p%	log β	p%	log β	p%	log β	p%	log β	p%	log β	p%	log β
5	–	0.25	–	0.21	–	0.11	78	0.10	78	0.14	78	0.12
10	–	0.16*	–	0.18	–	0.13	77	0.11	78	0.12	77.5	0.12
20	–	0.16	–	0.15	–	0.12	78	0.12	79.5	0.09	79	0.11
30	–	0.23	–	0.19	–	0.12	–	–	–	–	–	–
40	–	0.20	–	0.17	–	0.20	80	0.19	78.5	0.16	79	0.18
50	–	0.20	–	0.18	–	0.18	–	–	–	–	–	–
60	–	0.17	–	0.18	–	0.18	79.5	0.17	81	0.17	80	0.17
70	–	0.18	–	0.19	–	0.17	–	–	–	–	–	–
80	–	0.17	–	0.15	–	0.15	78.5	0.19	78.5	0.16	78.5	0.17
90	–	0.19	–	0.16	–	0.16	–	–	–	–	–	–
100	–	0.17	–	0.14	–	–	76	0.13	78	0.14	77	0.14
110	–	0.12	–	0.16	–	–	–	–	–	–	–	–
120	–	0.16	–	0.15	–	–	74.5	0.06	76	0.11	75	0.09
130	–	–	–	0.16	–	–	–	–	–	–	–	–
140	–	–	–	0.18	–	–	75.5	0.05	74	0.09	75	0.07
150	–	–	–	0.15	–	–	–	–	–	–	–	–
160	–	–	–	0.18	–	–	74	0.04	75.5	0.08	75	0.06
170	–	–	–	0.12	–	–	–	–	–	–	–	–
180	–	–	–	0.08	–	–	75.5	0.03	73.5	0.06	74.5	0.04
190	–	–	–	0.09	–	–	–	–	–	–	–	–
200	–	–	–	0.10	–	–	75.5	0.02	74	0.04	75	0.03
210	–	–	–	0.06	–	–	–	–	–	–	–	–
220	–	–	–	0.05	–	–	74.5	0.03	75.5	0.04	75	0.03
230	–	–	–	0.05	–	–	–	–	–	–	–	–
240	–	–	–	0.08	–	–	77.5	0.01	75.5	0.06	76.5	0.03
245	–	–	–	0.04	–	–	–	–	–	–	–	–

* Repeated samples at this same position and depth gave the following data:

Time:	8:10 p.m.	8:20 p.m.	8:30 p.m.	8:40 p.m.	8:50 p.m.	9:00 p.m.	9:10 p.m.
log β:	0.16	0.17	0.12	0.14	0.15	0.13	0.16

of the photomultiplier used (Du Mont Type 6292) was sufficiently high that signal levels in extremely clear water were not far above this background when the polarizing prism was at right angles to the predominant *e*-vector.

Results. The *in situ* measurements of scattering and polarization at Stations 1, 3 and 5 are summarized in Tables 12, 13 and 14 and in Figures 15 and 16. Note that only log β measurements are given in the first columns at all three Stations because the too rapid rotation of the prism had not been realized at that time. Also it should be pointed out that the scattering coefficient of the calibrating glass cube used in the *in situ* experiments was 0.75 smaller than that for the cube used in water sample measurements. Consequently all the present determinations were multiplied by 0.75 to bring them into line with the previous ones.

Discussion. Several differences should be noted between the *in situ* measurements and the laboratory determinations on water samples. To begin with the reproducibility of the results appears better *in situ*. Thus variation in log β is less than 0.05 in the sea but in water samples may vary, as pointed out above, from 0.08 in turbid water to greater than 0.2 in clear samples. However, the improvement in p's reliability is less definite in measurements made in the sea.

The relations between *in situ* measurements and those from samples are different for the inshore and offshore stations. The turbid inshore waters showed log β to have the same order of magnitude when measured in the sea and in the laboratory

TABLE 13. In situ *polarization and scattering (90°) at Station 3 (21/8/58)*

Depth (m)	Sample 1		Sample 2		Sample 3		Averages	
	p%	log β	p%	log β	p%	log β	p%	log β
0	–	1.45	–	–	38	1.42	38	1.43
5	–	1.45	37.5	1.49	39.5	1.44	38.5	1.46
10	–	1.49	38	1.54	37.5	1.52	37.5	1.52
12	–	1.52	37.5	1.48	38	1.49	37.5	1.50
14	–	1.57	35.5	1.52	–	–	35.5	1.54

(Tables 8, 10, 13 and 14). However, the degree of polarization was appreciably higher (46–48%) in laboratory samples than *in situ* (38%). The reason for this discrepancy is not certain, but it may have been due either to large particles which settled out in the samples or to the influence of background illumination produced by the *in situ* meter. This should be tested by designing another instrument which would not be subject to the latter alternative.

At Station 1 the scattering coefficients *in situ* were distinctly smaller than those in the laboratory (Tables 6 and 12) whereas the polarization *in situ* was greater (75–80%) than it was in the laboratory (60%). These discrepancies are probably largely due to the effects of, 1) contamination of the samples, 2) alteration due to warming or aging, and 3) presence of some scattered light in the laboratory meter.

Examination of the correlation of p and log β in the average *in situ* data of 12/9/58 at Station 1 (Fig. 17) indicates that suspended particles with a strong effect on polarization were present above 100 m and more particularly between 20 and 80 m. The fact that these particles produce a slight increase in the scattering coefficient between 40 and 80 m is of particular interest since increased p is usually associated with decreased β. The observed effect of the particles in question may provide a means for their future identification.

Another point of interest in this p–log β diagram is that the data above 100 m roughly fit a straight line with a slope different from that of the straight line of best fit for the data below 120 m. This is consistent with the hypothesis proposed above that in water samples the slope of the p–log β dia-

TABLE 14. In situ *polarization and scattering (90°) at Station 5*

Depth (m)	21/8/58		13/9/58	
	p%	log β	p%	log β
0	–	1.57	–	–
2	–	1.52	–	–
3	–	–	38	1.52
4	–	1.57	–	–
5	–	–	38	1.54
6	–	1.58	–	–
7	–	–	39	1.54
8	–	1.57	–	–

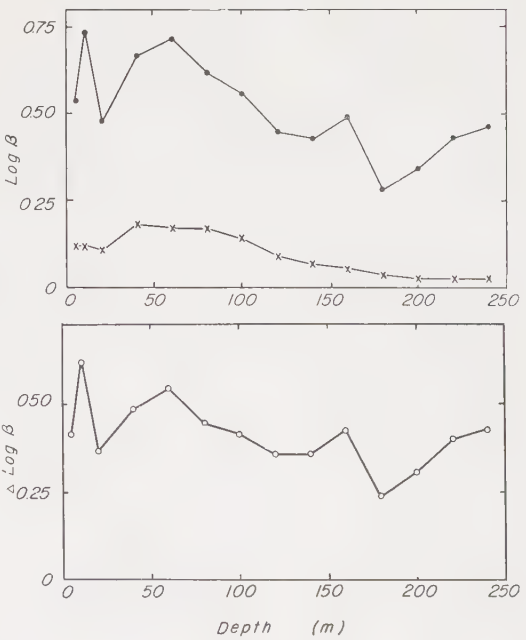

FIG. 15. Comparison of log β's at Station 1 obtained with measurements on water samples and *in situ*. The filled circles show this parameter as a function of depth measured as samples taken 12/9/58 while the crosses are average log β determined *in situ* on the same day. The open circles show the difference (Δ log β) between the results obtained by the two methods.

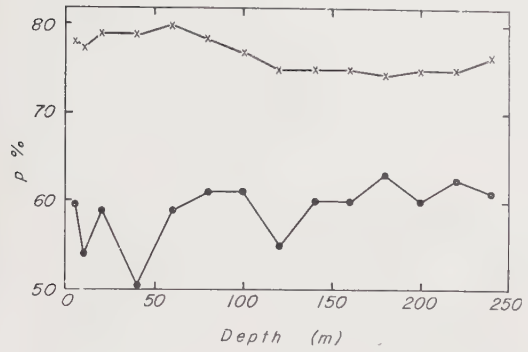

FIG. 16. Comparison of p%'s at Station 1 obtained with measurements on water samples and *in situ*. The filled circles show the parameter as a function of depth as measured on water samples taken 12/9/58 while the crosses are average degrees of polarization determined *in situ* on the same day.

gram varies with the nature of the suspended particles.

A final matter of interest is the relation between the present measurements of polarization and those made previously on natural underwater polarization at the same stations (Ivanoff and Waterman 1958b). Data for such a comparison are assembled in Table 15 where p_d is the degree of polarization of radiant energy from the sun and sky at 9 m measured using a narrow band green filter horizontally *in situ* at 90° to the sun's bearing. In the next column p_a is the degree of polarization at 10 m measured in the present observations on nearly parallel beams of artificial light *in situ* at Station 1 and in the laboratory for Stations 3, 4 and 5. The ratio p_a/p_d shows that natural underwater polarization decreases more rapidly than the degree of polarization of the water with a nearly parallel beam as the turbidity increases. This results from the dependence of p_d not only on the suspended particles but also on the directionality of the submarine irradiation which decreases as turbidity is increased (Waterman 1954; Ivanoff 1957).

TABLE 15. *Comparison of polarization data for daylight and for a beam of artificial light*

Station	p_d for daylight	p_a for artificial light	p_a/p_d	log β
1	50%	78%	1.6	0.12
3	20%	50%	2.5	1.3
4	23%	52.5%	2.3	1.0
5	9%	49%	5.5	1.6

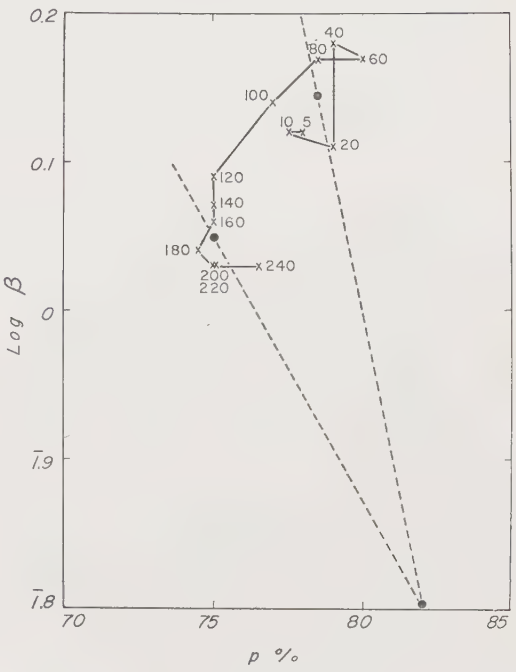

FIG. 17. Averaged data obtained *in situ* at Station 1 on 12/9/58 and plotted as a p–log β diagram. The filled circle at $p = 82\%$, log $\beta = -0.2$ represents approximately pure water (see Fig. 12). The filled circle at $p = 78.5\%$, log $\beta = 0.145$ is the average data at depths less than 100 m, while the filled circle at $p = 75\%$, log $\beta = 0.05$ is the average of the measurements made at more than 120 m. The two straight broken lines thus have slopes of

$$\frac{0.145 + 0.2}{0.82 - 0.785} = 9.9 \quad \text{and} \quad \frac{0.05 + 0.2}{0.82 - 0.75} = 3.6 .$$

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DIURNAL VARIATIONS IN SOME CHEMICAL AND BIOLOGICAL PROPERTIES OF THE SARGASSO SEA[1]

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ABSTRACT

Periodic measurements were made during a 24-hr period in the surface layer (0–300 m) of the Sargasso Sea off Bermuda of the following variables: temperature, salinity, phosphate, nitrite, nitrate, chlorophyll, C^{14} assimilation and zooplankton standing crop. Nutrient concentrations, integrated from the surface to 150 m, increased at night and decreased during the day. Chlorophyll concentrations increased during the daylight hours and decreased at night. C^{14} assimilation over 24-hr periods was greater in experiments initiated at night than in those begun in the daytime. Thus changes in the C^{14}/chlorophyll relationship appear to have resulted from variations of both parameters. Zooplankton standing crop (0–200 m) increased markedly during the early evening but subsequently decreased throughout the rest of the night and following day.

Short-term diurnal variations in plant nutrients, phytoplankton chlorophyll, and photosynthetic potential have been reported by several investigators (Doty and Oguri 1957; Yentsch and Ryther 1957; Shimada 1958; Park *et al.* 1958; Bruce and Hood 1960). Seldom, however, have these different variables been observed simultaneously in such studies, and never in the open sea.

The reported variations in the photosynthetic potential were observed by the authors by measuring C^{14} uptake of surface samples taken at different times of the day and placed under constant artificial illumination. On an oceanographic cruise between Bermuda and the West Indies during January–February, 1960, a somewhat different type of photosynthetic periodicity was observed. Productivity measurements were made at 22 stations both by a simulated *in situ* C^{14} technique and by the chlorophyll-radiation method of Ryther and Yentsch (1957). By standard physical and chemical criteria, the surface waters of the area investigated were very similar and primary production, as determined by both methods showed little variability (Ryther and Menzel, *in press*). Nevertheless there was a strong indication that C^{14} uptake in experiments which were begun at night tended to be higher than in those initiated during the daylight hours, despite the fact that all of these measurements were made over a 24-hr period and under natural illumination. This is a rather different situation from that previously reported in which the instantaneous photosynthetic potential, under constant, artificial illumination, was found to vary diurnally. On the other hand, chlorophyll and hence the productivity values calculated from chlorophyll and radiation, were somewhat higher when the former was sampled during the day and lower when sampled at night.

These results, while suggestive, were far from conclusive. Not only did the ship cover a large area geographically, where conditions may have varied significantly, but the "day–night" interpretation is an oversimplification of the fact that most of the daytime stations were occupied in the early morning and most of the night stations early in the evening. To substantiate these findings, it was obviously necessary to make observations at the same location around the clock. To explain the behavior, if observed, corollary measurements of as many related environmental factors as possible were also clearly indicated.

On April 27–28 the authors spent 24-hr aboard the R. V. PANULIRUS at a location 15 miles southeast of Bermuda in 1500 fathoms of water. Earlier studies at this sta-

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tion (Station "S") indicated that conditions there were essentially those of the open Sargasso Sea (*cf.* Menzel and Ryther, 1960). Observations were made every 4 hr at selected depths to 300 m, and the following measurements were made: temperature, salinity (using a conductivity meter constructed at the British National Institute of Oceanography), phosphate (Murphy and Riley 1958), nitrite (Rider and Mellon 1946), nitrate (Mullin and Riley 1955) and chlorophyll, to 150 m only (Richards with Thompson 1952; Creitz and Richards 1955). Primary production was measured by the C^{14} technique (Steemann Nielsen 1952) on samples taken from the depths to which 100, 50, 25, 10 and 1% of the incident radiation penetrated. These samples, with C^{14} added, were incubated for 24 hr in a water-cooled box under neutral density filters transmitting the same fraction of the incident light to which the water was naturally exposed. Due to space limitation in the incubator, these measurements were made only four times, at 1330, 2100, 0030 and 0800 hr, respectively. Incident radiation was measured with an Epply pyrheliometer and this value and the chlorophyll data were used to calculate primary production by the method of Ryther and Yentsch (*loc. cit.*). Samples of zooplankton were obtained by making vertical hauls from 200 m to the surface with a #8 one-meter plankton net.

The complete results of our study, with the exception of the zooplankton data, are given in Table 1. The observations were

TABLE 1. *Physical, chemical and biological data, 0–300 m, in the Sargasso Sea off Bermuda at six times during a 24-hr period on April 27–28, 1960*

	Depth (m)	T (°C)	Sal. (‰)	σ_t	NO_2–N μg A/L	NO_3–N μg A/L	PO_4–P μg A/L	Chl *a* μg/L	Carbon/assim/m³/day	
									C^{14}	Chl–Rad.
Time: 1330	0	21.30	36.69	25.70	.010	.09	.08	.04	4.1	0.9
	15	20.36	36.62	25.92	.008	.29	.07	.05	3.7	1.6
	25	20.28	36.61	25.94	.008	.14	.05	.06	2.1	1.7
	50	19.58	36.60	26.11	.016	.07		.09	0.6	1.3
	75	19.25	36.59	26.19	.021	.17	.05	.31		
	100	18.82	36.58	26.29	.126	.10	.05	.54	0.4	0.6
	125	18.76	36.58	26.31	.024	.34	.09	.16		
	150	18.72	36.58	26.32	.010	.40	.11			
	200	18.54	36.57	26.36	.040	.31	.10			
	300	18.03	36.49	26.43	.016	.80	.18			
Time: 1600	0	21.06	36.64	25.74	.003	.27	.10	.13		3.0
	15	20.31	36.62	25.93	.010	.02	.07			
	25	20.23	36.62	25.95	.021	.08	.06			
	50	19.52	36.60	26.13	.008	.07	.07	.12		1.8
	75	19.37	36.58	26.15	.008	.16		.22		
	100	18.89	36.58	26.28	.024	.04	.06	.81		0.9
	125	18.76	36.60	26.32	.042	.34	.06	.10		
	150	18.70	36.59	26.33	.018	.45	.09	.07		
	200	18.60	36.58	26.35	.029	.60	.12			
	300	18.07	36.49	26.42	.005	1.73	.21			
Time: 2100	0	21.45	36.65	25.64	.021	.22	.07	.05	5.8	1.2
	15	20.39	36.61	25.90	.008	.09	.07	.05	4.2	1.6
	25	20.31	36.60	25.92	.008	.08	.06	.08	2.8	2.3
	50	19.74	36.60	26.06	.005	.08	.09	.08	0.6	1.2
	75	19.51	36.60	26.12	.003	.17	.07	.09		
	100	19.15	36.57	26.20	.008	.16	.07	.40	0.5	0.5
	125	18.76	36.57	26.30	.137	.20	.10	.65		
	150	18.69	36.57	26.32	.024	.54	.13	.12		
	200	18.53	36.56	26.36	.029	.98	.10			
	300	18.00	36.46	26.42	.021	.84	.25			

TABLE 1 (*Continued*)

	Depth (m)	T (°C)	Sal. (‰)	σ_t	NO_2–N μg A/L	NO_3–N μg A/L	PO_4–P μg A/L	Chl *a* μg/L	Carbon/assim/m³/day C^{14}	Carbon/assim/m³/day Chl–Rad.
Time: 0030	0	21.54	36.62	25.59	.008	.08	.11	.04	4.3	0.9
	15	20.39	36.60	25.89	.003	.12	.07	.05	3.4	1.6
	25	19.87	36.59	26.02	.016	.25	.07	.05	2.8	1.4
	50	19.40	36.59	26.15	.003	.12	.07		1.9	
	75	19.01	36.58	26.24	.013	.09	.06	.24		
	100	18.84	36.58	26.29	.047	.28	.09	.54	0.6	0.6
	125	18.77	36.57	26.30	.032	.22	.09	.31		
	150	18.72	36.57	26.31	.024	.27	.14	.06		
	200	18.63	36.57	26.33	.024	.94	.09			
	300	18.15	36.49	26.40	.010	2.18	.11			
Time: 0400	0	21.00	36.62	25.74	.003	.09	.13	.04		0.9
	15	20.43	36.61	25.89	.003	.15	.09	.05		1.6
	25	20.22	36.63	25.96	.008	.07	.04	.04		1.1
	50	19.27	36.58	26.18	.003	.09	.09	.10		1.5
	75	19.00	36.58	26.25	.008	.11	.06	.41		
	100	18.84	36.58	26.29	.108	.30	.07	.45		0.5
	125	18.75	36.57	26.30	.029	.33	.10	.20		
	150	18.75	36.57	26.30	.023	.84	.14			
	200	18.65	36.57	26.34	.010	1.55	.11			
	300	18.40	36.53	26.36	.013	1.15	.13			
Time: 0800	0	20.98	36.61	25.74	.021	.12	.07	.07	5.0	1.7
	15	20.74	36.61	25.80	.008	.20	.06	.06	4.3	1.9
	25	20.02	36.60	25.99	.018	.30	.05	.06	1.5	1.7
	50	19.34	36.58	26.16	.010	.10		.12	0.9	0.8
	75	18.89	36.58	26.28	.042	.24	.06	.19		
	100	18.78	36.58	26.31	.016	.20		.27	0.9	0.3
	125	18.72	36.57	26.31	.029	.87	.09	.05		
	150	18.69	36.57	26.32	.008	.94	.10	.08		
	200	18.60 ?	36.56	26.34	.028	.33	.17			
	300	18.35 ?	36.53	26.37	.018	.53	.11			

made following the brief spring diatom flowering which is typical of the region (Hulburt *et al.* 1960). Summer conditions had just set in with the surface waters thermally stratified, nutrient impoverished, and sparsely populated with microorganisms. The greatest density of phytoplankton, as indicated by chlorophyll concentrations, occurred at about 100 m, at or near the compensation depth and at the point where the nutrient level begins to increase with depth. Again this situation is representative of summer conditions in the area (Menzel and Ryther *loc. cit.*).

It is under these impoverished conditions that one might expect the greatest response of organisms to daily changes in the environmental factors which critically limit their production. On the other hand, the concentrations of nutrients and phytoplankton and the level of primary production were all so low as to approach the limit of sensitivity of the various methods. For this reason it is difficult to detect and evaluate minor fluctuations in these variables. This is particularly true for individual measurements, but when the quantities and rates are integrated over the depth of the euphotic zone, within which several measurements were made, random errors tend to be cancelled out and a more reliable picture of changes with time is obtained.

Measurements of the extinction coefficient with a submarine photometer revealed that 1% of the incident radiation penetrated to approximately 100 m. We have integrated the nutrient and chlorophyll values to 150 m since the density remained al-

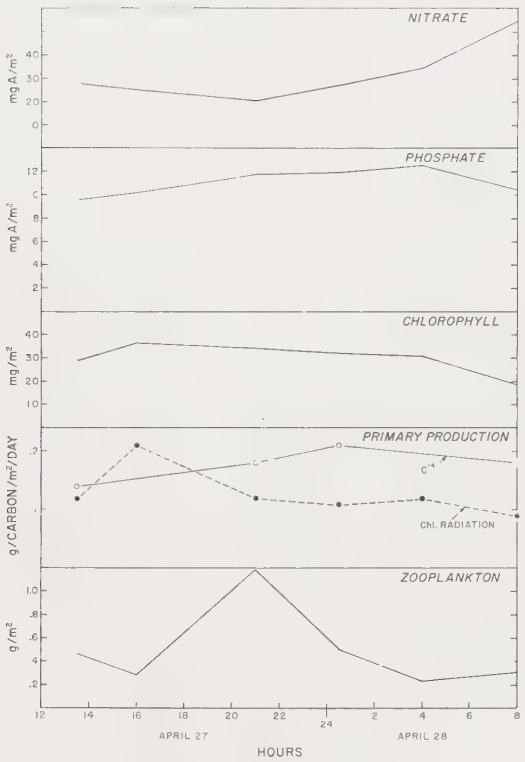

FIG. 1. Diurnal variations in nitrate, phosphate, chlorophyll, primary production and zooplankton standing crop at Station "S", April 27–28, 1960.

most constant at that depth (see Table 1) indicating that there were no appreciable effects of internal waves or other hydrodynamic features. Photosynthesis values were integrated to 100 m, below which our previous investigations have shown no detectable carbon assimilation. The zooplankton standing crop to 200 m was, of course, integrated over that depth by the method of collection. The results are shown in Figure 1.

Integrated nitrite variations were extremely small and irregular with time; hence they are not illustrated. It is noteworthy, however, that nitrite maxima were observed at 100–125 m ($\sigma_t = 26.29 - 26.30$) at the same depth as the chlorophyll maximum, confirming the observations of Vaccaro and Ryther (1960), who attribute this correlation to nitrate reduction by phytoplankton at low light intensities. This nitrite maximum varied from 0.042 to 0.137 μgA/L, but it is difficult to say whether these variations represent diurnal changes or whether the maximum occurs in such a narrow depth band that it was not sampled each time with the same precision.

The concentrations of nitrate and phosphate integrated over 150 m showed significant and systematic changes with time. Both nutrients were higher at night than during the day, but the nitrate reached its highest level at 0800, after increasing throughout the night, while phosphate began to increase in the afternoon, reached a peak at 0400 and decreased between 0400 and 0800 with a periodicity quite similar in time and magnitude to that described by Bruce and Hood (*loc. cit.*). Nitrate ranged from 20 – 55 mgA/m^2 within the depth studied while phosphate varied only from 9.6 – 12.6 mgA/m^2. The differences between the maximum and minimum concentrations of nitrate and phosphate were 35 mgA/m^2 NO_3–N and 3 mgA/m^2 PO_4–P respectively, a ratio of 12:1. If the phytoplankton assimilate carbon, nitrogen and phosphorus at a ratio of 100:15:1 by atoms (*cf.* Redfield 1934), the calculated daily rate of carbon fixation from the change in nitrate and phosphate would be 2.8 and 3.6 g/m^2 respectively, figures an order of magnitude higher than the observed production rates reported below. Apparently the exchange of these elements between organisms and water are far more rapid than and independent of the carbon cycle.

The chlorophyll increased during the day to a peak of 37 mg/m^2 at 1600 and then decreased during the night to a minimum of 19 mg/m^2 at 0800. Productivity calculated from these chlorophyll values and the radiation for the 24-hr period following the time of sampling, by the method of Ryther and Yentsch (1957) were therefore higher during the day when the chlorophyll concentrations were higher, and lower at night, ranging from 0.09–0.23 gC/m^2/day. The C^{14} uptake values, on the other hand, behaved in the opposite way. Though fewer measurements were made, the lowest value (0.12 gC/m^2/day) was obtained in the experiment initiated at midday, the highest (0.21 gC/m^2/day) at midnight. These re-

sults, then, confirm those found by Ryther and Menzel (*loc. cit.*). Since the two methods of estimating production vary independently and oppositely, changes in their mutual interrelationship cannot be attributed to an inconsistency of either method alone. The diurnal increase and nocturnal decrease in chlorophyll is similar to that described in a small polluted estuary (Ryther *et al. loc. cit.*). The most likely explanation is a direct effect of light on the pigment or its synthesis. Why the daily uptake of C^{14} should depend upon the time within the 24-hr cycle that the experiment was started is more difficult to explain. It is clear that there is a daily cycle of available nutrients, but what role, if any, this plays remains obscure.

The standing crop of zooplankton was measured with the thought that the nocturnal migration of animals into the euphotic zone could influence the nutrient concentrations, by their excretions, or the phytoplankton populations, by their grazing activities. Such influences are nowhere obvious. The zooplankton behaved in a curious manner, increasing in abundance by fivefold in the upper 200 m between 1600 and 2000, but subsequently becoming less numerous throughout the night and showing no correlation with either nutrients or chlorophyll.

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FURTHER INVESTIGATIONS ON THE CARBOHYDRATE CONTENT OF MARINE ZOOPLANKTON[1]

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ABSTRACT

Studies on the carbohydrate content of 11 species of planktonic animals have been carried out. In the case of *Calanus finmarchicus, C. hyperboreus* and *Neomysis americana,* certain animals were fed laboratory phytoplankton cultures and comparisons were made between fed and starved groups. For *Calanus finmarchicus,* fed animals contained from 0.90–2.85 μg and starved animals from 0.68–1.50 μg carbohydrate/animal. As percent wet weight the carbohydrates ranged from 0.11–0.40 for fed and from 0.10–0.24 for starved *C. finmarchicus.* The carbohydrates in *C. hyperboreus* ranged from 0.14–0.29% wet weight and in *Neomysis* from 0.19–0.23%, but there was no significant difference between fed and starved animals. Feeding experiments were also attempted with the euphausids *Meganyctiphanes norvegica* and *Thysanoessa* sp. but feeding was poor and again no significant difference over the concentrations in starved animals could be detected. The concentration for all the euphausids was rather low: 0.05–0.08% wet weight for *Meganyctiphanes,* 0.09–0.11% for *Thysanoessa,* and 0.08–0.17 for *Nematoscelis megalops.* Carbohydrates were also low for the copepods *Paraeuchaeta norvegica* (0.06–0.11%) and *Pleuromamma* sp. (0.10%) and for *Hyperia galba* (0.15%). Another amphipod *Euthemisto* contained from 0.25–0.62% wet weight carbohydrate and an ostracod *Conchoecia* 0.31%. Respiratory measurements during periods of starvation were made for *Calanus hyperboreus, Nematoscelis megalops* and *Neomysis americana* which indicated that considerably more carbon was being oxidized than could be accounted for by changes in the carbohydrate content of the animals. Conversely, in certain feeding experiments with *Neomysis americana* and *C. finmarchicus* it seemed very doubtful that sufficient carbohydrate was obtained in the food consumed to meet the carbon requirements if carbohydrate was the principal energy source. It is concluded that the animals in question do not contain sufficient carbohydrate reserves to meet energy requirements, particularly during periods of food scarcity, and therefore, other metabolic substrates must be utilized as well.

INTRODUCTION

An understanding of the metabolism of the zooplankton is important to the study of food chains and energy flow in the sea. Although it is well known that the nature of the substance oxidized, whether carbohydrate, fat or protein, has a marked influence on the amount of oxygen used in the respiratory processes, very little work on the composition of the zooplankton organic matter has been carried out to date. Carbohydrates, in particular, are poorly known and the results of previous investigations have been widely divergent. The carbohydrate contents suggested by Brandt (1898) and Brandt and Raben (1919), for example, for mixed plankton are approximately ten times the values found in recent preliminary studies by Raymont and Krishnaswamy (1960) on selected species of zooplankton. These studies on zooplankton from the English Channel have been extended in the present investigation to some neritic species off the northeastern United States, and to several deep-water species. Since it had been shown also by Raymont and Krishnaswamy that starvation tended to lower carbohydrate levels in plankton, it was desirable to supplement the work on carbohydrate content with some investigations on respiration and feeding rates in the same species.

One of us, (J.E.G.R.) desires to express his thanks to the Director of the Woods Hole Oceanographic Institution for the invitation to work at the Institution and for a grant covering the period of the investiga-

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Thanks are due Dr. John Barlow, Masateru Anraku, Michael Mullin, and Thomas Renshaw who aided in the collection of the experimental material. Dr. Robert Guillard kindly supplied the phytoplankton used in the feeding experiments. Constance Chadwick assisted in the identification of certain of the zooplankton species.

METHODS

The zooplankton used in the experiments were collected with a coarse plankton net (#00 or #000) in Cape Cod Bay, Gulf of Maine (42°10′N, 69°45′W) and also from the slope water off the eastern tip of Georges Bank (41°40′N, 65°05′W) and south of Woods Hole (39°N, 71°30′W). The catches were diluted and kept cool on board ship, and after being brought to the laboratory they were further diluted and kept in a cold room at 5°C. They were sorted as soon as possible into polyethylene vessels containing one to two liters of filtered sea water and maintained in a refrigerator at about 5°C.

Determinations in which comparisons were made on carbohydrate levels in fed and unfed animals were carried out on *Calanus finmarchicus*, and a few similar data were obtained for *Calanus hyperboreus* and *Neomysis americana*. The diatoms *Skeletonema costatum*, *Thalassiosira fluviatilis*, and *Rhizosolenia setigera* and an unidentified flagellate designated *Olisthodiscus* I, all from the Woods Hole Oceanographic culture collection, were used in the feeding experiments. Food concentrations were determined by cell counts using a Levy counting chamber with Fuchs Rosenthal rulings. In addition to changes in the cell numbers during the course of feeding experiments, the number of fecal pellets produced was also used as an index to the amount of feeding in some instances.

Feeding experiments were also attempted with two carnivorous forms, *Paraeuchaeta norvegica* and *Euthemisto compressa-bispinosa*[2] using living and dead copepods and portions of euphausids. However, feeding was limited for *Euthemisto* and *Paraeuchaeta* apparently did not eat any of the food offered it.

A few carbohydrate determinations were performed on other species of zooplankton, even though it was not possible to compare the effects of feeding and starvation satisfactorily. Included in this group were the euphausids *Thysanoessa* sp., *Meganyctiphanes norvegica*, *Nematoscelis megalops*, the amphipod *Hyperia galba*, the ostracod *Conchoecia* sp. and the copepod *Pleuromamma* sp.

Respiratory rates were determined for *Calanus hyperboreus*, *Nematoscelis megalops*, and *Neomysis americana* using the Winkler method as employed by Conover (1956, 1959) and from these rates, the amount of carbohydrate which could have been oxidized during the experiments was calculated.

For the determination of carbohydrates it was necessary to use about 100 *Calanus finmarchicus* (mostly stage V with a few females) for a single analysis, but much smaller numbers of larger zooplankton animals were required for a determination (e.g., only a single *Meganyctiphanes*). In each case the animals were rinsed briefly in distilled water, excess liquid removed with filter paper and then weighed. The methods of Mendel, Kemp and Myers (1954) and Kemp and Kits van Heijningen (1954) were used for the determination of carbohydrate, the animals being first ground with a mixture of 5% trichloracetic acid, containing 0.1% Ag_2SO_4, and the mixture then boiled for 15 min. After cooling and centrifuging, 1 ml of the supernatant liquid was boiled for 6½ min with 3 ml concentrated H_2SO_4 (1.84 specific gravity). The intensity of the resulting pink color was then determined by a Beckman D.U. spectrophotometer at 520 mμ, the readings be-

[2] Both *Euthemisto compressa* and *E. bispinosa* are found in the Gulf of Maine, often together (Bigelow 1925). The animals are quite similar in many respects and no attempt was made to distinguish between them in this study.

TABLE 1. *Carbohydrate content, grazing rate, and fecal pellet production of* Calanus finmarchicus *(mainly stage V copepodites)*

Date of haul	Date of analysis	Expt.	Number of animals	Carbohydrate content		Grazing rate ml/ copepod day	No. fecal pellets produced	Remarks
				µg/ copepod	% wet wt			
28.VII	29.VII	A_0	100 V	1.50	0.24	–	–	Initial, starved 24 hr
28.VII	30.VII	AT_1	99 V	1.26	0.18	–	2,200	Starved 24 hr, fed 20 hr
28.VII	30.VII	AT_2	97 V	1.39	0.20	–	1,932	Starved 24 hr, fed 21 hr
28.VII	30.VII	AS_1	98 V	1.89	0.29	–	4,730	Starved 24 hr, fed 22 hr
28.VII	30.VII	AS_2	94 V	1.70	0.24	–	6,110	Starved 24 hr, fed 23 hr
28.VII	30.VII	AC_1	82 V	1.22	0.18	–	281	Starved 38 hr
28.VII	30.VII	AC_2	c. 100 V	(1.10)*	0.16	–	318	Starved 39 hr
28.VII	30.VII	AC_3	84 V	1.07	0.14	–	390	Starved 41 hr
28.VII	30.VII	AC_4	c. 100 V	(0.85)*	0.14	–	252	Starved 42 hr
28.VII	3.VIII	B_1	200 V	1.23	0.18	6.9	9,160	Starved for 5 days, then fed 16 hr
28.VII	3.VIII	B_2	200 V	0.68	0.10	–	1,000	Starved for 6 days
3.VIII	4.VIII	$CR_{7 \text{ and } 8}$	100 V	1.45	0.19	–	1,000	Starved for 21 hr
3.VIII	4.VIII	$CR_{9 \text{ and } 10}$	100 V	2.45	0.28	29.8	6,760	Fed for 21 hr
3.VIII	6.VIII	$DR_{1 \text{ and } 2}$	100 V	2.70	0.38	–	5,760	Fed for 23 hr
3.VIII	7.VIII	$DR_{3 \text{ and } 4}$	100 V	2.85	0.40	–	9,360	Fed for 47 hr
3.VIII	7.VIII	$DR_{5 \text{ and } 6}$	106 V	(2.10)*	(0.26)	–	5,320	Fed for 23 hr, then accidentally killed
3.VIII	12.VIII	$ER_{4 \text{ and } 5}$	100 V	0.90	0.11	av. 15.6	3,041	Starved for 10 days, fed 18 hr
3.VIII	13.VIII	$ER_{1 \text{ and } 2}$	100 V	1.15	0.15	av. 17.0	2,680	Starved for 10 days, fed 45 hr
3.VIII	13.VIII	$ER_{3 \text{ and } 6}$	100 V	1.25	0.17	av. 17.0	1,998	Starved for 10 days, fed 24 hr, then starved 22 hr
25.VIII	26.VIII	F_3	117 IV & V	1.07	0.16	–	–	Starved for 1 day

* Values omitted from subsequent calculations

ing converted into units of glucose from a calibration curve. This method gave fairly accurate determinations for values from about 10 to about 110 µg glucose, but for higher amounts it was necessary first to dilute the supernatant fluid before boiling with H_2SO_4. In any case, several replicates were run for each sample and these generally agreed within one or two percent. Some difficulty was experienced on a few occasions owing to the necessity of using a different Beckman spectrophotometer with slightly variable sensitivity but a later check showed that the values were of the right order of magnitude and that apparently little error was introduced. For each analysis the amount of carbohydrate has been expressed as percentage of the wet body weight.

RESULTS

For *Calanus finmarchicus* the carbohydrate content of several sets of fed and starved animals were compared (Tables 1 and 2). The results show a considerable range of variability (0.11 – 0.40% for fed animals and 0.10 – 0.24% for starved), but for each set of experiments with one exception the data are reasonably consistent.

In series A (used almost immediately after capture on July 28, 1959, in Cape Cod Bay) there was a decided lowering of the carbohydrate levels in animals starved for 38 to 42 hr ($AC_1 - AC_4$) in comparison with the initial A_0. Among fed aminals AT_1, AT_2, AS_1 and AS_2 the levels remained high. Animals AT_1 and AT_2 received 2,190 cells/ml of *Thalassiosira fluviatilis* as food

TABLE 2. *Carbohydrate content of fed and starved* Calanus finmarchicus *(summary of data in Table 1)*

	All experiments on fed and unfed *Calanus* except $DR_{5 \text{ and } 6}$				Experiments on *Calanus* kept in laboratory not more than 7days *i.e.*, excluding series E			
	Carbohydrate % wet wt		Carbohydrate/ copepod (μg)		Carbohydrate % wet wt		Carbohydrate/ copepod (μg)	
	Range	Mean	Range	Mean	Range	Mean	Range	Mean
Fed	0.11–0.40	0.23	0.90–2.85	1.71	0.18–0.40	0.27	1.23–2.85	1.93
Starved	0.10–0.24	0.16	0.68–1.50	1.16	0.10–0.24	0.16	0.68–1.50	1.16

while animals AS_1 and AS_2 received 96,200 cells/ml of *Skeletonema costatum*. *Thalassiosira* is a substantially larger diatom than *Skeletonema* and the concentrations were adjusted to provide approximately the same biomass of cells per unit volume in each feeding culture. Nonetheless, *Calanus* produced twice as many fecal pellets when fed *Skeletonema* as when fed *Thalassiosira* and the carbohydrate levels in the AS_1 and AS_2 groups would seem to be significantly higher than in AT_1 and AT_2.

Fecal pellet production is not always a reliable index of the amount of food consumed unless some account is taken of the fact that pellets vary in size over a wide range. In the B series of experiments about 1,000 fecal pellets were produced by the starved B_2 animals. However, these pellets were very small and contained almost no particulate matter. On the other hand, the B_1 animals, which had a substantially higher carbohydrate content than the B_2 group, produced over 9,000 large, green pellets.

Both A and B animals were captured in the same tow-netting on July 28, 1959. If the A_0 group contains the initial concentration of carbohydrate at time zero, the amount of carbohydrate for starved animals in the groups AC_1, AC_3 and B_2 falls very roughly along a straight line when plotted on semi-logarithmic paper against time of starvation. Assuming that the loss of sugars is explained by a single logarithmic function, it can be calculated that the daily loss was about 13% for AC_1, 19% for AC_3 and 14% for B_2.

Three additional experiments, series C, D and E, were carried out with animals taken on August 3, 1959, in Cape Cod Bay. In series C, animals fed *Thalassiosira fluviatilis* ($CR_{9 \text{ and } 10}$) were compared with animals $CR_{7 \text{ and } 8}$ which were not fed (see Table 1). The D group was fed a mixture of the diatom *Th. fluviatilis* and a flagellate, possibly *Olisthodiscus* sp. In this experiment animals fed for varying periods of time were compared but unfortunately animals $DR_{5 \text{ and } 6}$, which were fed for one day and then starved, were accidentally killed by some contaminated water during the starvation period. Nonetheless, in both C and D experiments the carbohydrate content would seem to have increased as a result of feeding. The marked fecal pellet production in $CR_{9 \text{ and } 10}$, $DR_{1 \text{ and } 2}$, and $DR_{3 \text{ and } 4}$, indicating considerable feeding, is in agreement with the higher levels of carbohydrate observed.

The results from the experiments E are generally inconsistent. The animals used here had been kept under starvation conditions for 10 days and presumably they were in poor condition at the start of the run. Their carbohydrate content was lower than in most of the other experiments, even after two days of feeding. Fecal pellet production was not so great as in some other runs.

Another attempt to get *C. finmarchicus* from Cape Cod Bay on August 25 was largely unsuccessful. Only enough animals including some stage IV were obtained to run a single analysis F_3.

It is possible, since known numbers of *Calanus* were employed for the analyses, to calculate the carbohydrate content per copepod. The corrected values, omitting the experiments where animals were kept for more than one week, suggest substantially more carbohydrate in fed (mean 1.9 μg) as compared with starved copepods (mean 1.2 μg) (Table 2). These values may be compared with those obtained by Raymont and Krishnaswamy (1960) for *Calanus* from

TABLE 3. *Carbohydrate content and respiratory rate for* Calanus hyperboreus. *Copepods from haul 7.VIII.58, 42°10′N, 69°45′W*

Date of analysis	No. and stage of copepods	Carbohydrate content		Respiratory rate		Carbohydrate equivalent, μg glucose/animal day	Grazing rate ml/animal day	Remarks
		μg/copepod	% wet wt	μL/animal day	μL/mg wet wt day			
10.VIII	15 V	11.7	0.21	–	–	–	–	Not fed
15.VIII	20 V	8.2	0.16	–	–	–	–	Starved 7 days, fed 1 day
15.VIII	17 ♀	29.4	0.29	–	–	–	–	Starved 7 days, fed 1 day
17.VIII	20 V	9.2	0.18	–	–	–	3.71 (*Skeletonema*)	Starved 7 days, fed 3 days
17.VIII	20 V	12.7	0.20	–	–	–	2.57 (*Skeletonema*)	ditto
17.VIII	20 V	8.8	0.16	–	–	–	2.03 (*Skeletonema*)	ditto
17.VIII	18 ♀	15.8	0.18	–	–	–	4.25 (*Skeletonema*)	ditto
17.VIII	19 V	7.9	0.16	9.9	2.11	13.0	–	Starved 7 days, fed 1 day before expt., then starved 2 days
17.VIII	20 V	7.4	0.14	10.7	2.03	14.0	–	ditto
17.VIII	20 V	7.5	0.14	7.9	1.47	10.4	–	ditto
17.VIII	18 ♀	29.7	0.29	17.4	1.70	22.8	–	ditto

the English Channel. For freshly caught and for fed copepods they found a mean carbohydrate content of 1.30 μg; for starved *Calanus*, a value of 0.89 μg per copepod.

The data for *Calanus hyperboreus* (Table 3) suggest a carbohydrate content very similar to that for *C. finmarchicus*. Enough females of this species were taken to permit separate analysis. For experiments on stage V the mean carbohydrate value was 0.17% of the wet weight. For females, the result is more variable: two of the three determinations gave a value of 0.29% but one was much lower; the mean for all analyses was 0.25%. In any event the value suggested for female *C. hyperboreus* is of the same order as that for stage V and VI *C. finmarchicus*. Expressed as the amount of carbohydrate per copepod, the values for *C. hyperboreus* (stage V) ranged from 7.4 μg to 11.8 μg (mean 9.1 μg). For females the value is somewhat doubtful, as again one experiment gave much lower results than the other two, 15.8 μg as against 29.4 μg and 29.7 μg (Table 3). It is clear, however, that the larger female specimens have a considerably greater amount of carbohydrate than stage V copepodites but it would appear probable that the percentage of carbohydrate to body weight is also somewhat greater in females as compared with stage V of *C. hyperboreus*.

There appears to be little effect on carbohydrate level with feeding and starvation in *C. hyperboreus* but the data are few and uncertain. The mean level in fed stage V copepodites was 0.18% wet weight and for starved 0.15%. For the females, the starved animals appeared to contain more carbohydrate than the fed (Table 3). The grazing rate resulting from these experiments was very low. A mixture of *S. costatum* and *Th. fluviatilis* was given the animals but only the former was eaten. Subsequent work on *C. hyperboreus* has demonstrated that one to two weeks of exposure to laboratory conditions may be necessary before any consistent feeding occurs. Therefore, it is not surprising that differences in amount of sugar between fed and starved animals were not great. Since all animals had been starved for nearly a week before the experiments, the levels of carbohydrate were probably somewhat lower than in nature.

Some attempts were made to compare fed and starved euphausids, *Meganyctiphanes norvegica* and *Thysanoessa* sp., captured east of Georges Bank in the slope water. In the case of *Meganyctiphanes* the carbohydrate content was low in all analyses and there is no evidence that the level was affected by the food given (Table 4). All the experimental animals were kept without food for 5 days, but after feeding

TABLE 4. *Carbohydrate content, respiratory rate and grazing rate for some euphausids*

Animal	Date captured	Expt. date	Carbohydrate content		Respiratory rate		Carbohydrate equivalent, μg glucose/animal day	Grazing rate, ml/animal day	Remarks
			μg/animal	% wet wt	μl/animal day	μl/mg wet wt day			
Meganyctiphanes norvegica	6.VIII	14.VIII	153	0.05	–	–	–	–	Starved 5 days, fed 3 days
"	6.VIII	14.VIII	99	0.05	–	–	–	–	ditto
"	6.VIII	14.VIII	233	0.06	–	–	–	–	ditto
"	6.VIII	14.VIII	114	0.04*	–	–	–	–	ditto
"	6.VIII	14.VIII	213	0.04*	–	–	–	–	ditto
"	6.VIII	18.VIII	151	0.05	–	–	–	55.1 *Skeletonema*	Starved 5 days, fed 7 days
"	6.VIII	18.VIII	130	0.05	–	–	–	25.7 *Thalassiosira*	ditto
"	18.VIII	21.VIII	128	0.08	–	–	–	–	Starved 3 days
Thysanoessa sp.	6.VIII	18.VIII	72	0.11	–	–	–	62.3 *Skeletonema* 25.7 *Thalassiosira*	Fed 7 days
"	6.VIII	18.VIII	68	0.11	–	–	–	42.9 *Skeletonema* 38.3 *Thalassiosira*	Fed 7 days
"	18.VIII	21.VIII	16	0.09	–	–	–	–	Starved 3 days
Nematoscelis megalops	6.VIII	14.VIII	135	0.13	–	–	–	–	Starved 5 days, fed 3 days
"	18.VIII	18.VIII	53	0.12	–	–	–	–	–
"	18.VIII	18.VIII	–	0.17	–	–	–	–	–
"	18.VIII	18.VIII	50	0.15	–	–	–	–	–
"	18.VIII	18.VIII	82	0.13	–	–	–	–	–
"	18.VIII	18.VIII	50	0.13	–	–	–	–	–
"	18.VIII	18.VIII	56	0.15	–	–	–	–	–
"	18.VIII	18.VIII	51	0.11	–	–	–	–	–
"	18.VIII	19.VIII	46	0.13	93.5	2.54	122.8	–	No food 10°C
"	18.VIII	19.VIII	71	0.08	112.2	3.12	147.3	–	
"	18.VIII	19.VIII	50	0.14	140.5	2.63	184.5	–	
"	18.VIII	19.VIII	60	0.13	159.3	3.42	209.2	–	No food 4°C
"	18.VIII	19.VIII	45	0.13	116.8	3.23	153.4	–	
"	18.VIII	19.VIII	49	0.12	97.9	2.57	128.5	–	

* Animals dead before analysis

the monosaccharides and glycogen levels were still less than in one animal which was taken in a haul from slope water on August 18 and kept for 3 days without food (Table 4). A grazing rate computed for two animals kept in 900 ml of culture containing initially 6600 cells/ml of *Thalassiosira* and 119,900 cells/ml of *Skeletonema* was not particularly large when it is considered that the animals had an average wet weight of 285 mg, more than two orders of magnitude greater than *C. finmarchicus*. As in the experiment with *C. hyperboreus*, *Skeletonema* was favored over *Thalassiosira*, but it is probable that animal food is a necessary supplement to the diet of *Meganyctiphanes*.

The carbohydrate level in *Thysanoessa* was somewhat higher than in *Meganyctiphanes* and possibly affected to a greater extent by feeding on laboratory cultures of phytoplankton. A grazing rate was obtained for two groups; these specimens had only 25% of the biomass of *Meganyctiphanes* but had a similar filtration rate (Table 4). The carbohydrate content of these fed *Thysanoessa* was 0.11% of the wet weight. Another group of animals starved

TABLE 5. *Carbohydrate content, respiratory rate, and grazing rate for* Neomysis americana. *All animals obtained from Cape Cod Bay 25.VIII.1959*

Date of analysis	Expt.	No. of animals	Carbohydrate content		Respiratory rate		Carbohydrate equivalent, μg glucose/animal day	Grazing rate, ml/animal day	Remarks
			μg/animal	% wet wt	μl/animal day	μl/mg wet wt day			
26.VIII	M_1	15	16.7	0.23	–	–	–	–	Initial
26.VIII	M_2	15	17.3	0.22	–	–	–	–	Initial
27.VIII	M_3	14	15.0	0.20	47.8	6.37	62.8	–	No food, 4°C
27.VIII	M_4	14	14.3	0.20	46.0	7.01	60.4	–	No food, 4°C
27.VIII	M_5	14	12.5	0.21	54.9	9.25	72.1	–	No food, 10°C
27.VIII	M_6	14	13.9	0.20	60.0	8.47	78.8	–	No food, 10°C
27.VIII	M_7	15	13.0	0.19	–	–	–	7.7	Fed *Skeletonema costatum*, 4°C
27.VIII	M_8	15	11.7	0.22	–	–	–	43.0	Fed *Skeletonema costatum*, 4°C

over the same period had a carbohydrate content of only 0.06% wet weight. This latter value has, however, been omitted from Table 4, since the animals were dead or at least moribund before the analysis.

Additional analyses were performed on a few *Meganyctiphanes* and *Thysanoessa* taken in the slope water at about 39°N, 71°30′W on August 18. The animals had been without food for a few days so that the observed values were probably a little lower than expected for fresh material. It will be noted that these *Thysanoessa* were much smaller than the previous group and could have been juveniles or another species.

Despite the few determinations it is probable that the normal carbohydrate level for these two species of euphausids is probably in the vicinity of 0.10% of wet weight or somewhat lower than that observed for *C. finmarchicus* in Cape Cod Bay and in the English Channel (Raymont and Krishnaswamy 1960). The third species of euphausid studied, *Nematoscelis megalops* may contain somewhat more carbohydrate than the others tested (Table 4). The mean value of carbohydrate was 0.13% and the range was not excessively great (0.08 to 0.17%). To some extent this may reflect the fact that almost all determinations were made on one haul of *Nematoscelis* from the slope water south of Woods Hole and that the animals were kept only one day before analysis. Nevertheless, three groups of the animals were kept at a somewhat higher temperature (10°C) which might have been expected to deplete carbohydrate reserves, and one determination was made on a different group of animals which were kept for over one week and only received food for the last 3 days (Table 4). The carbohydrate content in both these experiments, however, was virtually the same as in the other analyses. Although carbohydrate is probably slightly higher in *Nematoscelis* than in the other two euphausids, it still appears to be somewhat below that of copepods such as *C. finmarchicus* from Cape Cod Bay. It would be of interest to learn whether in *Nematoscelis* the carbohydrate level fluctuates appreciably with food supply.

A few experiments made on the mysid *Neomysis americana*, taken in Cape Cod Bay, showed fairly constant carbohydrate values. The amount of glucose per mysid varied between 12 μg and 17 μg but the size of animal used differed considerably so that the values when expressed as percentage of wet weight varied only from 0.19% to 0.23% (Table 5). The mean for all determinations, including animals analyzed within one day of capture, as well as those fed and those starved in the laboratory was 0.21%. The results available suggest that there was practically no effect on carbohydrate level with feeding on phytoplankton for 20 hr or starvation over the

TABLE 6. *Carbohydrate content of certain zooplankton species*

Date of capture	Date of analysis	No. of animals	Carbohydrate content		Remarks
			µg per animal	% wet wt	
			Paraeuchaeta		
7.VIII	13.VIII	11 V	3.2	0.07	
7.VIII	13.VIII	12, mostly ♀	9.2	0.10	
7.VIII	20.VIII	30 V	3.7	0.07	Little, if any, food taken
7.VIII	20.VIII	25 ♀	8.0	0.06	
18.VIII 7.VIII	20.VIII	15 ♀	13.3	0.11	
			Euthemisto		
7.VIII	11.VIII	7	>110*	>0.25*	
7.VIII	12.VIII	4	128	0.33	Some food consumed
7.VIII	13.VIII	4	166	0.35	
18.VIII	21.VIII	10	28	0.62	
			Pleuromamma		
18.VIII	21.VIII	94 V & VI	1.5	0.10	Not fed
			Hyperia		
7.VIII	20.VIII	10	24	0.15	Not fed
			Conchoecia		
18.VIII	21.VIII	50	7.5	0.31	Not fed

* Reading on spectrophotometer beyond limit of accuracy and sample lost before a dilution was measured.

same period. The mean carbohydrate percentage given for the species *N. americana* (0.21%) agrees well with that previously quoted by Raymont and Krishnaswamy (1960) for *Neomysis integer* from Plymouth, in which fed animals had a mean carbohydrate level of 0.26% of the wet body weight. Previous results with *N. integer*, however, suggested that on some occasions starvation reduced the carbohydrate reserves.

A number of additional observations on the carbohydrate content of other zooplankton animals is shown in Table 6. The carnivorous copepod, *Paraeuchaeta norvegica*, was not observed to eat during these experiments, though it has been observed to eat small copepods on other occasions. The amount of sugars expressed as a percentage of wet weight was among the lowest observed (mean for all experiments 0.08%). Whether a well fed *Paraeuchaeta* fresh from the sea would have an appreciably higher content cannot be stated at this time. A single analysis performed on a group of *Pleuromamma*, mostly stage V and adult, suggested a carbohydrate level near or slightly lower than that observed for *C. finmarchicus*. However, the animals had had no food for 3 days prior to extraction.

Two species of hyperid amphipod known to be carnivorous, *Euthemisto compressabispinosa* and *Hyperia galba* were also examined. *Euthemisto* was given some animal food which it appeared to eat. The results (Table 6) suggest a distinctly higher level of carbohydrate exceeding that for the copepods, euphausids and mysids investigated. The percentage of carbohydrate was about 0.34% for two sets of animals which had been in the laboratory for 5 to 6 days, but a higher carbohydrate level exceeding 0.6% of the wet body weight was observed in one group of *Euthemisto* which had been kept for only 3 days in the laboratory. The remarkable difference in the amount of carbohydrate per animal (28 – 166 µg) is explained by the fact that the size of the animals used showed an enormous range in the case of this species. Only a single anal-

ysis was made on *Hyperia* and the amount of carbohydrate was much lower (0.15% wet weight). These *Hyperia* had been kept more or less without food for nearly two weeks, however, and it is possible that a recently captured *Hyperia* would have shown a carbohydrate level nearer to that of *Euthemisto*.

The single analysis performed on *Conchoecia* gave a relatively high level of carbohydrate (0.31% of wet weight).

DISCUSSION

It is clear from the preceding data that, among the animals examined here, the presence of a considerable reserve of stored carbohydrate is unlikely. To be sure, some species such as *C. finmarchicus* have a carbohydrate content which varies over rather wide limits while in the case of the euphausids analyzed, the data though probably not conclusive, suggest very little variation. All the animals with the possible exception of *N. americana* are pelagic. It is well known that the distribution of planktonic communities is far from uniform in the sea, and there are marked seasonal variations so that the diets available to these animals probably vary from virtual starvation to vast excess. To some extent pelagic animals must be able to take advantage of the periods of excess to store up food reserves for survival during periods of food scarcity.

In some of these experiments, respiratory rates were obtained for the zooplankton under conditions of starvation at the same time that changes in the carbohydrate content were being determined. If the amount of oxygen taken up by the animals is converted to its carbohydrate equivalent (glucose), it is seen for *C. hyperboreus* (Table 3), *N. megalops* (Table 4) and *N. americana* (Table 5) that several times the total carbohydrate in the animal should have been oxidized assuming a respiratory quotient equal to one, but practically no change in total carbohydrate was observed. It is unfortunate that no respiratory data were obtained for *C. finmarchicus* which showed the greatest variability in carbohydrate reserves among the animals tested. However, if respiration values at 5°C are taken from Marshall and Orr (1958) and converted to carbohydrate oxidized, the amount burned is about 6 μg per day per animal. The average amount of glucose for fed animals is, however, less than 2 μg per copepod, so that once again the daily requirement for respiration is much greater than the total quantity of carbohydrate available. Further, in experiments with starving *Calanus* the greatest daily loss in carbohydrate amounted to about 0.10% of the wet weight. The measurements on Cape Cod Bay *Calanus* made during the past summer suggest an average wet weight of about 0.7 mg per individual. Hence, the maximum carbohydrate loss measured in these experiments is nearly an order of magnitude smaller than the amount predicted from respiration experiments.

In the present series of experiments the content of readily available carbohydrate of a mass culture of *Skeletonema costatum*, the most commonly eaten phytoplankton organism, was also determined to be about 6 μg per 10^6 cells. Hence, in the experiments in which grazing rates were computed it is possible to compare the amount of carbohydrate theoretically oxidized with the maximum carbohydrate which might have been acquired in feeding. The best combination of respiratory and grazing data was obtained for *N. americana*. It can be shown in the case of M_7 and M_8 (Table 5) that 28×10^4 and 120×10^4 cells respectively were consumed over a period of a day during which time roughly 60 – 80 μg of carbohydrate could have been burned ($M_3 - M_6$, Table 5). Even in the case of M_8, which had the higher grazing rate, the theoretical maximum carbohydrate which it could have acquired was 7.2 μg or one tenth of the amount oxidized.

Even the grazing rate observed for the mysids (43 ml/animal/day, Table 5), however, is not particularly large compared with values given in the literature for other animals. *N. americana* used for these studies was about 10 times as large on a wet weight basis as the *C. finmarchicus* used. Yet the

grazing rate computed for *Calanus* in Table 1 was in one instance 70% that of the mysids and never as low as 10%. Grazing rates for *Calanus* from 70 ml to over 200 ml/day have been reported in the literature (Gauld 1951; Harvey 1937). Raymont and Krishnaswamy (1960) found that *N. integer* was cannibalistic in laboratory culture. Possibly *N. americana* would feed more readily if there was some animal protein available.

It is regrettable that grazing rates were not computed for the A *Calanus* series in Table 1 since the increase in carbohydrate with feeding on *Skeletonema* for AS_1 and AS_2 would seem to be significant if the level for A_0 is taken as a base line. However, the fecal pellet production can be used to give a rough estimate of the number of cells consumed. From the B series and E series where both cell counts and fecal pellet counts were made for *Calanus* feeding on *Skeletonema*, an average number of cells per fecal pellet can be determined. (The data for the C series could not be used as *Th. fluviatilis* was the food organism in this case.) The computed values varied from 7,600 cells/fecal pellet to 25,300 with a mean value of 15,800. Using this mean value it can be shown that the AS_1 animals consumed about 762×10^3 cells during the experiment and the AS_2 about $1{,}027 \times 10^3$ cells. With the carbohydrate content for *Skeletonema* mentioned above, carbohydrate income assuming 100% assimilation was about 4.5 μg for AS_1 and 6.2 μg for AS_2. For B_1 the carbohydrate uptake would have been around 5 μg and for the E series it ranged from 1.6 to 4.2 μg/day.

The error in the above calculations is certainly considerable, but it appears that under these experimental conditions, despite the low content of readily available carbohydrate in *Skeletonema*, the intake of food would just about fulfill the daily carbohydrate requirement. However, in the laboratory experiments the cell concentrations fed to the animals were one or two orders of magnitude greater than would be commonly found even in rich coastal waters.

In this study the 6 μg of carbohydrate found per million cells of *Skeletonema* would be equivalent to about 2.4 μg of carbon. Brand (1935) found 45 to 67 μg of carbon per 10^6 cells in *Skeletonema* which suggests that carbohydrate makes up a relatively small percentage of the total organic matter in this diatom. Clearly a *Calanus* could eat considerably fewer than a million *Skeletonema* cells and meet its food requirements, if it utilized the other forms of carbon available in addition to carbohydrate. Of course, when fat and protein in addition to carbohydrate are burned, the carbon requirement per milligram of oxygen used in respiration is also reduced.

There is considerable danger in extending the results of laboratory studies to the open ocean without qualification. However, judging from the existing data the amount of carbohydrate-rich food in the ocean available to the herbivorous zooplankton is not always very great. In 15 analyses of net catches of naturally occurring diatoms and dinoflagellates performed by Brandt and Raben (1919) there was a considerable range in carbohydrate from 0–46%.[3] Conversion of carbohydrate to fat by the zooplankton seems only likely when carbohydrate is very plentiful as it is an energy-consuming process. The reverse conversion of fat to carbohydrate which is then oxidized, or direct oxidation of fat, requires more oxygen than the direct oxidation of carbohydrate, but over twice as much energy is produced per gram of fat oxidized as per gram of carbohydrate. Since oxygen is seldom limiting in the sea whereas organic carbon may be, among the zooplankton in impoverished areas a considerable portion of the metabolic substrate might logically be expected to consist of fat.

It may be that carbohydrates acquired in the food are oxidized directly by the

[3] Brandt and Raben (1919) determined the fat, protein, chitin and ash in their catches and estimated carbohydrate by difference. Therefore, included in their estimates must be some stable polysaccharides such as cellulose and carbohydrates in other combinations. It is still uncertain whether the herbivorous zooplankton can make use of such material. Water and glycerine extracts of *Calanus finmarchicus* showed no evidence for presence of a cellulase (Marshall and Orr, 1955).

zooplankton or are stored briefly in the same chemical form when there is an excess. On the other hand, fats may be oxidized directly when needed or stored as the principal food reserve. Thus a more coastal organism such as *C. finmarchicus* generally living at shallower depths in regions of relatively high organic productivity might be expected to have a rather variable carbohydrate content, while an organism from deep water or the open ocean such as *C. hyperboreus* or *P. norvegica*, and many euphausids might be expected to have a relatively constant carbohydrate content, which would be a function of the rate of conversion of fat to carbohydrate rather than the amount of food consumed.

Certainly the assumption that R.Q. = 1, frequently made by ecologists in trophic calculations, ought to be qualified in the future. Analysis of both fat and carbohydrate content on the same species of zooplankton, together with measurement of oxygen consumption, carbon dioxide production, and nitrogen excretion are essential for a complete picture of zooplankton metabolism, but the present studies form a preliminary contribution to the over-all problem.

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THE UPTAKE AND RELEASE OF INORGANIC PHOSPHORUS BY *DAPHNIA MAGNA* STRAUS

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ABSTRACT

Radio-chemical and chemical studies of the uptake of inorganic phosphorus by *Daphnia magna* have shown that an apparent direct uptake of 2.2×10^{-3} μg/animal/hr of PO_4·P from solution is caused by epizootic bacteria, which depend on organic substrates in the water for their activities. It is not known whether or not this phosphorus is available to *D. magna*.

D. magna loses inorganic phosphorus to the ambient medium at the rate of 8.4×10^{-3} μg/animal/hr. Loss of phosphorus is independent of epizootic bacteria and production of feces. No loss of organic phosphorus was detected.

Although the excretion of phosphatase by *D. magna* was confirmed, the regeneration of inorganic phosphorus due to hydrolysis of naturally-occurring organic phosphorus compounds is negligible compared with the direct release of inorganic phosphorus by *D. magna*.

INTRODUCTION

Tracer studies of the phosphorus circulation in the water of small lakes have demonstrated the extreme mobility of inorganic phoshorus (Rigler 1956). There is a rapid removal of phosphate from solution by plankton and an equally rapid release of phosphate into the water by plankton. Thus, measurements showing that the concentration of inorganic phosphate in the epilimnion remains constant over a few hours or days indicate, not that phosphate is unused, but that the rate of removal is exactly balanced by rate of regeneration. Temporal changes of phosphate concentration can be caused by a very slight difference between the two rates.

Although turnover of inorganic phosphorus is often caused primarily by bacteria and other nannoplankton it is important to define the position of other organisms in the phosphorus cycle. Zooplankton, for example, influence the phosphorus cycle in a number of ways. They have been observed to concentrate P^{32} (Coffin *et al.* 1949) probably by feeding on phytoplankton and thus remove phosphorus from the level of primary producers. However, assimilation is not complete (Marshall and Orr 1955) and 10–50% of ingested phosphorus is returned in fecal pellets. This may be returned to solution or lost as pellets sink from the epilimnion. It is also known that the phosphorus of dead zooplankton is rapidly returned to solution (Cooper 1935; Gardiner 1937) and it has been postulated that phosphatases released from living and dead zooplankton liberate inorganic phosphate from the soluble organic phosphorus compounds in the water (Steiner 1938; Margalef 1951).

The work reported in this paper is concerned with the least known effects of zooplankton on the circulation of phosphorus in lake water. Quantitative measurements were made of direct uptake and release of inorganic phosphorus by the planktonic crustacean, *Daphnia magna*, and an attempt was made to measure the hydrolysis of naturally occurring, organic phosphorus compounds in lake water by living *D. magna*.

Research was carried out at Atomic Energy of Canada Ltd., Chalk River, Ontario. Financial support was received from A.E.C.L. and through a research grant from the Ontario Research Foundation.

MATERIALS AND METHODS

D. magna used in experiments were cultured in 10-gal aquaria and fed daily with yeast or algal cells. Occasionally a small amount of Difco nutrient broth was added to stimulate bacterial growth. If males, or females with ephippia appeared in a culture, it was discarded and another started with approximately 20 mature females.

Mature females, with an average dry wt of 0.25 mg, were used in all experiments. Before use, they were tranferred through several baths of sterile water in order to reduce the number of yeast, algal or bacterial cells introduced into the experimental water. Since the animals had been starved for at least two hours, all but the posterior (dorso-ventral) portion of the mid gut was evacuated before they were used in an experiment. Consequently there were very few fecal pellets produced during an experiment.

An artificial Ottawa River water (A.W.) was prepared by adding inorganic salts to distilled water to give ionic concentrations similar to those in Ottawa River water in the vicinity of Chalk River, Ontario (Thomas 1952). One liter of A.W. contained:

$MgSO_4 \cdot 7\,H_2O$	20.3	mg
$CaCl_2 \cdot 2\,H_2O$	18.4	mg
$NaHCO_3$	7.3	mg
K_2SO_4	2.23	mg
NH_4NO_3	0.64	mg

The pH of this solution was 6.9–7.0 and the concentration of $PO_4 \cdot P$ was 0.001 to 0.01 ppm. All analyses for inorganic phosphorus were done by the method reported earlier (Rigler 1956).

Uptake of P^{32} was used to give a quantitative measure of uptake of inorganic phosphorus. Rate of uptake was determined from the radioactivity of groups of 50 specimens which had been immersed in water containing P^{32} for different lengths of time, usually 2, 4 and 6 hr. Uptake of inorganic phosphorus was calculated as follows:

$$\frac{\text{Av. } PO_4 \cdot P \text{ in solution } (\mu g/ml)}{\text{Av. } P^{32} \text{ in solution (cpm/ml)}} \times P^{32} \text{ uptake (cpm/animal/hr)}$$

This approximation was satisfactory since P^{32} in solution remained almost constant, P^{32} uptake by *D. magna* was linear and change of $PO_4 \cdot P$ in water was linear.

Net loss of inorganic phosphorus by *D. magna* was determined by chemical analyses of water.

Radioactive phosphorus was obtained from A.E.C.L. as carrier-free P^{32} in 0.005 N HCl. *D. magna*, before being assayed for P^{32}, were removed from experimental solution, rinsed for 15 sec in nonradioactive A.W., blotted gently and dried. Then, 10 individuals were evenly distributed over an area ⅝–¾ in. in diameter in the center of each counting tray. Since each sample consisted of 50–200 individuals, this procedure had the disadvantage of involving 5–20 separate determinations of radioactivity for each sample. The counting efficiency for a sample distributed in this manner was found to be almost identical with that for a sample distributed evenly over the whole tray. Hence the counts of samples of *D. magna* were considered to be of equal efficiency to those of water and suspended solids, samples of which covered the whole area of the tray.

P^{32} in suspended solids (largely bacteria) was determined by filtering 10 ml of water through an HA, Millipore filter and measuring radioactivity of residue on the filter. In some experiments there was almost no P^{32} in suspended solids and a considerable error was introduced by radioactivity of water absorbed by the filter. This residual radioactivity was found to be equal to that in 0.06 ml of water and has been subtracted from all results presented below.

Samples of total P^{32}, in suspended solids and in solution, were prepared by absorbing 2 ml of unfiltered water on a one-inch disc of filter paper on a counting tray. To prevent the filter paper from curling, drying by heat lamp was stopped when the filter paper was still damp. The sample was then allowed to dry slowly at room temperature.

Techniques for radioassay were the same as reported previously (Rigler 1956).

Specimens of *D. magna* were disinfected by immersion in A.W. containing 100 units/ml of penicillin and 100 μg/ml of streptomycin for two 90-min periods with an interval of 24 hr between the immersions. In order to determine the effectiveness of this method and to compare it with the method used by Harris (1957), the following test was carried out. One group (50) of *D. magna* were given two 90-min treatments at 24-hr intervals in A.W. containing 100 units of terramycin hydrochlo-

TABLE 1. *The numbers of viable bacteria associated with one* D. magna *before and after treatment with antibiotics*

Antibiotic used	Culture medium			Av.
	Alchem 299	Difco Nutrient Agar	Difco Heart Infusion Agar	
None	1.2×10^5	1.1×10^5	1.3×10^5	1.2×10^5
Terramycin	820	870	800	830
Streptomycin and penicillin	5	5	0	3

ride per ml neutralized with NaOH. It was necessary to neutralize the solution since terramycin hydrochloride reduced pH to 3.8 and caused 50% mortality of specimens treated. A second group was treated in the same way with the penicillin–streptomycin mixture and a third, control group, merely transferred through changes of sterile A.W. After treatment, each group was rinsed for 3 hr in a large volume of sterile A.W. to remove traces of antibiotics, ground in a glass homogenizer, suspended in distilled water and plated on several types of agar. Plates were incubated at 25°C for 48 hr and the number of colonies determined. The results, presented in Table 1, show that both methods reduce the number of viable bacteria greatly and that the streptomycin–penicillin mixture was far more effective than terramycin–hydrochloride. It was also observed that treatment of *D. magna* with neutralized terramycin caused most individuals to swim listlessly and erratically at the bottom of the container whereas the streptomycin–penicillin mixture has no obvious deleterious effect on behavior. For these reasons treatment with streptomycin and penicillin was retained as the method for reducing the number of microorganisms associated with *D. magna*.

Although the animals treated in this way were not bacteria-free, they carried less than 0.003% of their normal bacterial flora (Table 1). Hence it is considered that in the experiments described below the results obtained were the same as would have been obtained had sterile animals been used. Therefore the treated animals will be described as 'sterile' even though this is not strictly correct.

RESULTS

Uptake of Phosphate

Since bacteria which multiply rapidly in stored natural waters take up over 90% of P^{32} added to the water (Rigler 1956) and *D. magna* readily feeds on bacteria (Rodina 1940), direct uptake of phosphate by this animal must be measured in water containing no bacteria. This was done in the first experiment. Uptake of P^{32} from non-sterile Ottawa River water, filtered through Whatman #1 paper 24 hr before the experiment, was compared with uptake from sterilized water, filtered and autoclaved. Measurement of the amount of P^{32} in suspended bacteria showed that, in non-sterile water, 90% of added P^{32} was in bacteria throughout the experiment. In the autoclaved water, less than 2% of the P^{32} was taken up 3½ hr after the beginning of the experiment, but after this time the amount increased and at 8 hr, 15% of the P^{32} was in bacteria. Therefore, if uptake of P^{32} is considered only during the first 4 hr of the experiment, uptake from non-sterile water should give a measure of feeding rate and uptake from autoclaved water, a measure of direct uptake of phosphate from solution.

The results, presented in Figure 1, show that uptake of P^{32} was linear and was three times as rapid from non-sterile as from autoclaved water. Rate of uptake of P^{32} from non-sterile water in which suspended bacteria contained 134 cpm/ml was 333 cpm/animal/hr. If it is assumed that uptake of P^{32} by *D. magna* in this case was entirely due to ingestion of radioactive bacteria, then the filtering rate was 2.5 ml/animal/hr. The similarity between this value and filtering rates of 2–3.3 ml/animal/hr measured by Ryther (1954) suggests that the assumption is valid.

Uptake of P^{32} from autoclaved water was 110 cpm/animal/hr during the first 3½ hr when less than 3.6 cpm/ml of P^{32} had been taken up by suspended bacteria. In order to account for this rate of uptake by filtering of bacteria it would be necessary to postulate a filtering rate of 30 ml/animal/hr.

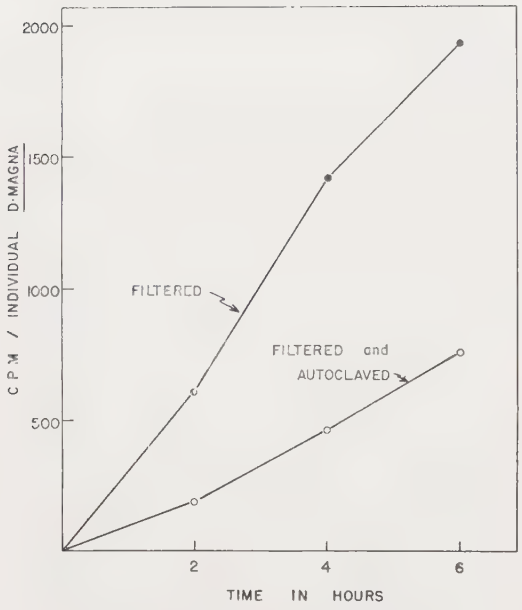

FIG. 1. Uptake of P^{32} by *Daphnia magna* from filtered and autoclaved, filtered Ottawa River water.

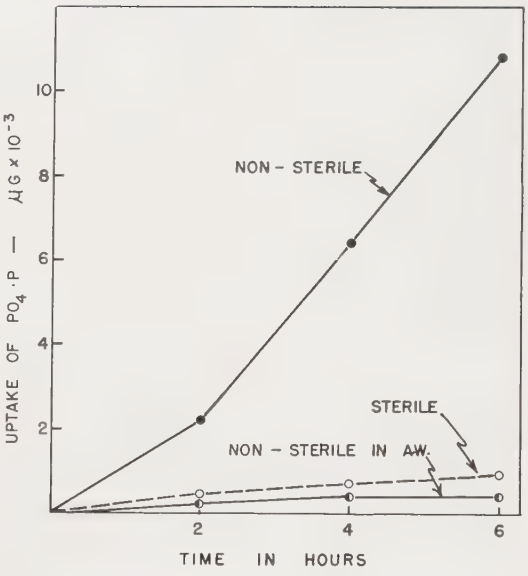

FIG. 2. Uptake of inorganic phosphorus by 1) non-sterile and 2) sterile *D. magna* from Ottawa River water, and by 3) non-sterile individuals from artificial Ottawa River water (A.W.) calculated from uptake of P^{32}. The average percentage of the P^{32} in suspended solids in each case was; 1) 0.9, 2) 0.3 and 3) 2.2.

Since this is 10 times greater than the maximum rate reported by Ryther (1954) and 7.5 times greater than the maximum rate of 4 ml/animal/hr observed in this laboratory (unpublished) it is most unlikely that uptake of P^{32} from autoclaved water was due to filtering of suspended bacteria. Some other mechanism whereby *D. magna* removes P^{32} from the water must exist and of the possible mechanisms the following appeared to be the most likely. 1) They might take up inorganic phosphate directly from solution (Marshall and Orr 1955). 2) Bacteria living in the gut or on the surface of *D. magna* might take up inorganic phosphate from solution. 3) Labelled phosphate might exchange with non-radioactive phosphate of organic compounds which are utilized by *D. magna*.

In order to determine which, if any, of the above mechanisms caused the uptake of P^{32} from sterile water, the following experiment was carried out. The uptake of P^{32} by sterile and non-sterile *D. magna* from river water sterilized by millipore filtration, and by non-sterile individuals from sterile A.W. was measured. The results (Fig. 2) show that prior sterilization of *D. magna* greatly reduces the uptake of phosphorus from sterile river water, and therefore that bacteria living on *D. magna* are responsible for the apparent uptake of PO_4 by this animal.

Comparison of uptake by non-sterile individuals in river water and in A.W. (Fig. 2) shows that the uptake was almost completely inhibited in A.W. Since A.W. differed from river water primarily in that it contained no organic matter, it is apparent that under conditions of this experiment (*D. magna* not feeding) the epizootic bacteria depend on organic material in the ambient medium for their activities.

This experiment also supplies more direct evidence that uptake of P^{32} from autoclaved river water in the first experiment was not due to a rapid filtering of suspended bacteria or particulate matter containing P^{32} by *D. magna*. Both the A.W. and river water containing non-sterile *D. magna* were slightly contaminated by bacteria introduced with the cladocerans. However, the uptake was slow in A.W., in which

TABLE 2. *Uptake of inorganic phosphorus by* D. magna *from synthetic Ottawa River water (A.W.) containing organic substrates*

Ambient solution	Phosphorus uptake (μg/animal/hr)	Percent of P^{32} in suspended solids
A.W.	1.7×10^{-4}	0.31
A.W. + 0.1% glucose	3.0×10^{-4}	0.44
A.W. + 0.1% asparagine	3.6×10^{-4}	0.71
A.W. + 0.1% asparagine and 0.1% glucose	4.2×10^{-4}	0.29

2.2% of the P^{32} was in suspended solids and fast in river water in which only 0.9% of the P^{32} was in suspended solids.

Although it had been shown that epizootic bacteria caused an apparent uptake of P^{32} by *D. magna* and that uptake did not take place from an inorganic solution, the possibility still remained that uptake of P^{32} represented, not a direct removal of inorganic phosphorus from solution, but a removal of organic phosphorus compounds with which $P^{32}O_4$ had exchanged. This appeared unlikely in view of the rapidity with which aquatic bacteria take up inorganic phosphate and the demonstration by Gourley (1952) that there is almost no exchange between inorganic phosphate and phosphate of organic phosphorus compounds dissolved in water. Nevertheless it seemed desirable to attempt to demonstrate a direct removal of inorganic phosphate from solution by epizootic bacteria. This was done by measuring uptake of phosphate from A.W. and from A.W. to which glucose and asparagine had been added. The results in Table 2 show that these organic substrates, either singly or together increased the rate of uptake of P^{32} from solution. In this case, no organic phosphorus compounds were present and uptake of P^{32} represented a direct uptake of inorganic phosphate.

The results of this experiment demonstrated only that a direct uptake of inorganic phosphorus is stimulated by organic substrates and not that all of the P^{32} taken up from sterile river water was in the form of inorganic phosphate. However, it seems reasonable, for the purpose of calculating direct uptake under normal conditions, to assume that this was the case. On this assumption an uptake of 43 cpm of P^{32}/animal/hr (Fig. 1) from water in which the phosphorus concentration was 0.004 ppm and the concentration of P^{32} was 180 cpm/ml represented an uptake of 2.5×10^{-3} μg $PO_4 \cdot P$/animal/hr. The uptake rate of 2×10^{-3} μg/animal/hr in Figure 2 was calculated in the same way.

Loss of Phosphate

An increase in the amount of inorganic phosphorus in water containing high concentrations of zooplankton has been observed and has been interpreted by Gardiner (1937), Cushing (1954) and Steele (1959) as indicating a direct excretion of inorganic phosphorus by zooplankton. However, Margalef (1951) has shown that living zooplankton secrete phosphatases into the water. Therefore the increases of phosphate observed, might have been caused, at least in part, by the hydrolysis of soluble organic phosphorus compounds. Another possible source of error causing results to be low would be the uptake of soluble phosphate by suspended and epizootic bacteria.

In the measurements reported below, excretion of phosphorus was calculated from analyses of water containing zooplankton, but precautions were taken to avoid the sources of error mentioned above. Possible hydrolysis of soluble organic phosphorus was avoided by using A.W. This also reduced uptake of inorganic phosphorus by epizootic bacteria to $1\text{–}1.7 \times 10^{-4}$ μg/animal/hr. (Fig. 2 and Table 2). Water was filtered before the experiments to remove suspended bacteria, and P^{32} was added to facilitate tests for uptake of inorganic phosphate by suspended bacteria. In none of the results reported was an error of more than 1% caused by suspended bacteria.

The results of all measurements, summarized in Table 3, show that the net loss of inorganic phosphorus is 8.3×10^{-3} μg/animal/hr. If a correction is applied for simultaneous uptake of inorganic phosphorus the gross loss is 8.4×10^{-3} μg/animal/hr.

TABLE 3. *Rate of loss of inorganic phosphorus by* D. magna

Time (hr)	Loss of PO_4·P ($\mu g \times 10^3$/animal/hr)* (1) Prestarved, in A.W.†	(2) Recently fed, in A.W.	(3) Sterile, in river water
0–1	9.0	9.9	8
1–2	8.1	8.0	
2–3	5.0	4.2	8
3–4	12	11	
4–5	–	–	9
5–6	7.8	–	
Average	8.3	8.1	8.3

† Average of four experiments.
* All experiments were done at 20–22°C, pH 7.0.

Further consideration of the data presented in Table 3 gives some insight into the source of the inorganic phosphate which is lost from *D. magna*. Firstly, it is suggested that the released phosphate is not associated with production of feces. This conclusion follows from the similarity between the rate of loss from animals which had been feeding until just before the beginning of the experiment (col. 2) and that from animals which had been starved for 3 hr before the experiment (col.1). It is further substantiated by the observation that the loss continued throughout 6 hr in spite of the fact that the animals were not feeding during the experiments. Secondly, the observation that the rate of loss of phosphate by sterile individuals (col. 3) was the same as from non-sterile individuals indicates that epizootic bacteria do not contribute to this loss. Therefore it is likely that the phosphate is lost in the copious urine which must be produced by this animal (Krogh 1939: p. 96).

Actually, the results presented above are not adequate to exclude bacteria as a possible source of phosphate. As was pointed out earlier, phosphatases secreted by *D. magna* might hydrolyze naturally occurring organic phosphorus and thus cause an increase of inorganic phosphate in natural waters containing these animals. If this were true a fortuitous balance between an increased liberation of phosphate by hydrolysis and a decreased liberation due to the absence of epizootic bacteria could have caused the similarity between the results in col. 3 and those in col. 1 and 2. In the next section it will be shown that this is not the case because under the conditions of the experiment reported in col. 3, no hydrolysis of naturally occurring organic phosphorus takes place.

Finally, in order to determine whether or not a significant amount of phosphorus was lost in combination with organic compounds, one series of measurements of excreted phosphorus was made in which total phosphorus as well as inorganic phosphate was measured. It was found that the average total loss of phosphorus was 6.0×10^{-3} μg/animal/hr and of inorganic phosphorus was 6.1×10^{-3} μg/animal/hr. Since it was obvious that if there were any loss of organic phosphorus it was very small compared with loss of inorganic phosphorus this aspect was not pursued further. However, interest attaches to the lower loss of phosphate in this than in previous experiments. A possible explanation is that it was carried out in artificial Toronto tap water which is very much harder than A.W., Ca and Mg being present in 50 and 7 mg/L respectively.

Release of Phosphatases by Living D. Magna

As was mentioned in the previous section, an apparently faster rate of loss of phosphate was expected in Ottawa River water than in A.W. due to hydrolysis of organic phosphorus compounds in the river water. This expectation was based on the work of Margalef (1951) which showed that living Cladocera hydrolyze sodium glycerophosphate in the ambient medium. However, the conditions of the experiment reported here differed in two ways from those in Margalef's work. Firstly the specimens used here were sterile whereas those used by Margalef were not; hence phosphatase activity measured by Margalef might have been due to bacteria. Secondly, the source of water used in the two cases was different and the waters may have differed in pH or amounts of various ions present. Thus the lack of phosphatase activity

in Ottawa River water might have been due to unfavorable conditions for the enzyme. Finally, extrapolation from experiments in which high concentrations of glycerophosphate are present to natural conditions is probably not justified since rate of hydrolysis at low concentrations of organic phosphorus found in nature (.01 – .02 ppm) may be very much lower (not higher as suggested by Steiner 1938) than rate of hydrolysis of 700 ppm used by Margalef. Also the organic phosphorus compounds in natural water are, perhaps, more resistant to enzymatic hydrolysis than is glycerophosphate.

In order to test these possibilities the following experiment was carried out. Four flasks, each containing one liter of sterile solution, were prepared. The first contained A.W., the second and third contained A.W. plus enough sodium glycerophosphate to give 1.0 ppm of phosphorus and the fourth contained sterile Ottawa River water plus the same amount of glycerophosphate. One hundred *D. magna* were placed in each solution. Those placed in the third and fourth flasks had been previously sterilized by treatment with antibiotics whereas those placed in the first and second were merely washed with A.W. The concentration of inorganic phosphate in each solution was measured at intervals over a 4-hr period and rate of increase of phosphorus was calculated.

The results of this experiment, presented in Table 4, show that in the three solutions containing glycerophosphate there was a large increase (average of 17×10^{-3} μg/animal/hr) in the rate of production of inorganic phosphate over and above the normal direct release of inorganic phosphate by *D. magna*. This increase can be attributed to hydrolysis of glycerophosphate by a phosphatase. Although there is a suggestion that rate of hydrolysis was more rapid in the solution containing non-sterile individuals (2) than in the flasks containing sterile individuals (3 and 4), enough hydrolysis occurred in the latter to demonstrate conclusively that the phosphatase activity can be attributed primarily to the zooplankton and not to their bacterial flora.

TABLE 4. *Rate of increase of soluble inorganic phosphorus in waters containing one* D. magna/*10 ml*

Specimens used‡	Ambient solution†	Increase of P in water ($\mu g \times 10^3$/animal/hr)	Increase due to hydrolysis of glycerophosphate ($\mu g \times 10^3$/animal/hr)
Non-sterile	(1) A.W.	7.8	0
	(2) A.W. + GP*	28	20
Sterile	(3) A.W. + GP*	24	16
	(4) River water + GP*	22	14

* Sodium glycerophosphate in concentration giving 1.0 ppm of phosphorus.
† 20°C; pH 7.0.
‡ Specimens used had been starved for 24 hours.

Table 4 also shows that there was a marked hydrolysis of glycerophosphate in Ottawa River water (14×10^{-3} μg/hr) which was very slightly less than that in A.W. (16×10^{-3} μg/hr). Consequently the hypothesis that Ottawa River water inhibits the phosphatase must also be rejected. It appears, therefore, that hydrolysis of naturally occurring organic phosphates does not take place, or takes place extremely slowly, because the substrate concentrations are very low or because the compounds present are not hydrolyzed by the enzymes released.

It was later shown that a sterile filtrate of water, which had contained high concentrations of *D. magna* for several hours, had 20–50% of the phosphatase activity (as measured by rate of hydrolysis of glycerophosphate) of the original solution and zooplankton. Attempts were made to demonstrate hydrolysis of naturally occurring organic phosphates by adding this sterile filtrate to sterile river water. This technique ruled out interference due to uptake and release of phosphate by zooplankton and due to uptake by bacteria so that the enzyme could be allowed to act for a longer time. Nevertheless, it was not possible to demonstrate a significant increase in concentration of inorganic phosphorus in experiments lasting as long as 48 hr.

DISCUSSION

When Coffin *et al.* (1949) added P^{32} to the surface of a small lake and observed that zooplankton concentrated it more than any other groups of organisms which they studied, they concluded that zooplankton "either take up phosphorus directly or feed with great rapidity on microorganisms that have absorbed the added phosphorus at once." It has since been shown by Rigler (1956) that, when P^{32} is added to lake water, microorganisms, particularly bacteria, do take up 95% of it almost at once and in this paper it is shown that *D. magna* accumulates P^{32} by feeding on these microorganisms. However, evidence concerning direct uptake of phosphorus is contradictory. On one hand Marshall and Orr (1955), using xenic *Calanus finmarchicus*, observed an uptake of P^{32} from sterile sea water; on the other, Harris (1957), using *Gammarus* sp., observed uptake from nonsterile water, but no uptake from sterile water. These apparently conflicting observations can, perhaps, be reconciled by the present work in which it is shown that *D. magna* possessing their normal bacterial flora take up phosphate from solution 20 times more rapidly than sterile individuals do. Marshall and Orr, who observed uptake of P^{32} from solution, did not sterilize their animals, whereas Harris, who did not observe uptake, sterilized his animals as well as the water in which they were placed. This suggests that the facilitated uptake observed in *D. magna* is perhaps a general phenomenon.

It still remains to be shown whether or not the phosphorus taken up from sterile lake water by *D. magna* becomes available to this animal. Tenuous evidence that at least part of the phosphorus may be utilized comes from the observation made by Marshall and Orr (1955), that *C. finmarchicus* placed in sterile water containing P^{32} produces radioactive eggs. However, there is no guarantee that animals as different as *C. finmarchicus* and *D. magna* take up P^{32} from sterile water by the same mechanism and hence that *D. magna* also utilizes the phosphorus. It it does, it must do so by digesting bacteria that have incorporated the phosphorus into their protoplasm. Since the bacteria must be attached to the exoskeleton, the only ones that can be eaten are those which break loose and are swept into the esophagus by feeding currents. Thus it is likely that, if any of the phosphorus taken up from solution is utilized, it can only represent a small part of the total.

Even if all of the phosphorus taken up directly were available to *D. magna* it would still be of little importance to the animal for direct loss of phosphorus is four times as rapid as direct uptake and must be made good largely with phosphorus from the food.

Nevertheless, direct uptake of phosphorus cannot be completely ignored for under some conditions it can be a source of significant error in experiments to measure the loss of phosphorus by zooplankton. In such experiments the water from a concentrated suspension of zooplankton is analyzed at intervals for inorganic phosphorus. The increase of phosphate is taken as a measure of the regeneration of phosphorus from zooplankton under natural conditions. But under the conditions of these experiments (*e.g.*, high organic content of water due to excretions of zooplankton), epizootic and suspended bacteria might flourish and take up inorganic phosphorus at a faster rate than reported here and hence cause the measured rate of excretion to be too low. As examples of measurements in which these sources of error were not considered, the work of Gardiner (1937) and Cushing (1954) can be cited. Both authors used the technique mentioned above to measure the rate of excretion of phosphorus by *Calanus finmarchicus* and their results differed by a factor of 10. Cushing (1959) suggested that the rate of excretion he measured was 10 times greater than that measured by Gardiner because his animals were larger and grazed more rapidly than Gardiner's. Steele (1959) also suggested that the difference could have been due to a difference in feeding rates and that Cushing's results could have been high because his animals were returning phosphorus to the water in partially digested fecal pellets. He also im-

TABLE 5. *Comparison of measured rate of excretion of phosphorus by* D. magna *and calculated rate of excretion by* C. finmarchicus

	D. magna	*C. finmarchicus*
Dry wt (mg)	0.25	0.3*
Phosphorus content (μg/animal)	0.5	3.2†
Phosphorus excreted (μg/hr/mg dry wt)	3.2×10^{-2}	4.8×10^{-2}
P excreted (%/hr)	1.6	0.45

* Marshall and Orr (1955b), p. 91.
† Calculated from figures collected by Vinogradov (1953).

plied that Gardiner's result was nearer to the expected rate of excretion. This may be true, but an alternative explanation is that a rapid uptake of phosphate by epizootic and suspended bacteria in Gardiner's experiments made his estimates of excretion rate much too low.

An indication that Cushing's results are not too high is given by the comparison of the rate of excretion by *C. finmarchicus* (Cushing 1954) and *D. magna* in Table 5. Relative to dry weight the excretion of phosphorus by *C. finmarchicus* is slightly higher, but relative to total phosphorus content of the animals the excretion rate of *D. magna* is higher. This direct comparison of excretion of phosphorus by *C. finmarchicus* with excretion by *D. magna*, although the former is a marine copepod and the later is a freshwater cladoceran, is more reasonable than comparison with mixed plankton used by Gardiner. Also since the *D. magna* used here were starving before and during the experiments, it seems unnecessary to postulate a rapid feeding of *C. finmarchicus* to account for an excretion rate similar to that by *D. magna*.

Excretion of inorganic phosphorus by zooplankton is of interest because it has been considered to be one of the major ways in which phosphorus is regenerated for further use by phytoplankton. (Steele 1959). It is now known that regeneration of phosphorus must be evaluated relative to the total regeneration, or turnover, since it has been shown by Rigler (1956) that there is a rapid turnover of inorganic phosphorus in lake water, and by Pomeroy (1960) that the turnover rate in sea water is almost as rapid as in lake water. But, no simultaneous measurements of total turnover rate and regeneration from zooplankton have been made and it is not yet possible to say whether or not the release of phosphate by zooplankton contributes significantly to the total regeneration. Nevertheless, a crude estimate can be made from available data if two unsupported assumptions are allowed. The first assumption is that the average of the rates of turnover of inorganic phosphorus (6 μg/L/hr) in the three lakes studied by Rigler (1956) is representative of the turnover rate in lakes generally. The second is that the rate at which planktonic animals excrete phosphorus is proportional to their size and hence that total excretion is proportional to the total weight of zooplankton. The dry weight of zooplankton per liter of lake water is estimated, from the data of Birge and Juday (1922), Davis (1958), and Pennak (1955), to be generally less than 1 mg. That is, it is less than the weight of 4 *D. magna* used in this investigation and hence, that the total excretion is less than 0.032 μg/L/hr. This is only 0.5% of the total turnover rate of 6 μg/L/hr. Thus it appears that the regeneration of phosphorus from zooplankton in lake water may prove to be a minor source of inorganic phosphorus.

Finally, this work has confirmed the observation made by Margalef (1951) that living Crustacea secrete phosphatase into the ambient medium. But it has not substantiated the suggestion of Steiner (1938), that phosphatases in natural waters cause a rapid decomposition of naturally occurring organic phosphorus compounds. Steiner based this prediction on the results of an experiment in which he observed the dephosphorylation of substrates added to water containing dead zooplankton because he felt that the rate of hydrolysis in his experiment was inhibited by accumulation of inorganic phosphorus. Contrary to this prediction, it has now been shown that, either due to the extremely low concentrations in which they occur or to their resistance to hydrolysis, the rate of hydrolysis of the naturally occurring phosphorus compounds is negligible. Thus it appears that,

just as direct secretion of phosphorus by zooplankton does not contribute significantly to regeneration of inorganic phosphates of lake water, neither does the effect of phosphatases secreted by these organisms.

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UPTAKE OF AMINO ACIDS BY MARINE INVERTEBRATES[1]

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ABSTRACT

The capacity for removal of amino acids from solution by marine animals was studied by following the concentration of an acid added to the ambient sea water. Thirty-five genera representing 11 phyla were examined. The representatives of 10 of the phyla removed significant quantities of glycine from solution during an observation period of from 16 to 24 hr. This capacity for uptake of glycine was not limited to filter-feeding animals. Observations using representatives of 3 phyla and various amino acids indicated that uptake could occur whether the acid concerned behaved as a cation or an anion at the pH of sea water. Comparison of the amino acid concentrations at the incurrent and excurrent siphons of the clam, *Spisula*, indicated that removal of glycine took place in the mantle cavity of this animal. Differences as great as 14% were observed. Disappearance of glycine at ambient concentrations as low as 1.5 mg/L could be demonstrated.

INTRODUCTION

Earlier workers suggested the possibility that organic material dissolved in sea water might serve as a significant source of food for aquatic animals, a hypothesis often associated with the name of Pütter (1909). Krogh (1931) in a critical review of the literature concluded that little evidence could be adduced to support this. In recent years, few studies have been directly concerned with this possibility. Collier, Ray, Magnitsky and Bell (1953) reported that an unidentified carbohydrate material was retained by oysters. Fox and his co-workers (1952, 1953) suggested that dissolved organic material may be adsorbed on particles of colloidal dimensions and become available to filter-feeding animals in this way. These workers also pointed out that the use of the terms "dissolved" and "particulate" has not been precise with respect to the organic matter in the sea. Utilization of such material remains an attractive idea since it can be demonstrated that in some cases the collection of phytoplankton by filter feeders does not provide sufficient material to support their growth and metabolism (Fox and Coe 1943).

It is apparent that the effective pore size of the filters used is of interest in determining potential food supplies. Most such studies (reviewed in Jørgensen 1955) indicate that particles of one to a few microns in diameter are at the lower limit for retention. However, there are some studies employing macromolecules which present notable exceptions to this statement. Thus MacGinitie (1945) studied the retention of proteins dyed with Evans Blue and concluded that the effective pore diameter of the mucus filters of *Urechis* and *Chaetopterus* was of the order of 40 A. Fox, Oppenheimer and Kittredge (1953) showed that *Mytilus* is capable of removing fish hemoglobin from solution.

It was of interest to determine whether small organic molecules were possibly available to marine invertebrates. Amino acids were selected because of their ease of estimation and because they were sufficiently small to make their physical restraint in the interstices of a filter improbable. It quickly became apparent that added amino acids could be removed by some of the animals employed and that this capacity was not limited to filter-feeding organisms. Consequently, this report is a survey of the capacity of marine invertebrates to remove amino acids from solution. Portions of this work have previously appeared in abstract form (Stephens and Schinske 1957, 1958).

MATERIAL AND METHODS

These observations were carried out on 35 genera of marine invertebrates represent-

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ing 11 phyla. The animals were collected in the vicinity of Woods Hole, Massachusetts and kept in the laboratory in running sea water for varying periods (not exceeding seven days) before being used.

Observations were made by adding a known amount of an amino acid to a volume of sea water in which the animals were then maintained. The concentration of the amino acid in the medium was monitored by periodic sampling and colorimetric estimation.

The concentrations of amino acids most frequently used in these studies were 2.0 mM and 1.0 mM. Stock solutions of 2.0 mM concentration were prepared and either used directly or diluted. Each set of observations included colorimetric estimation of the stock solution and dilutions thereof. For the most part, these stock solutions also contained 40 mg/L of tetracycline although streptomycin (55 mg/L) and penicillin (5×10^5 units/L) were used in some cases. These antibiotics were used to minimize bacterial breakdown of amino acids.

The volume of water in which the animal was placed varied from 50 ml to 1L depending on the size of the organism. Containers were placed in a bath of running sea water. No aeration procedure was carried out. Survival was excellent for periods of 72 to 96 hr.

The technique used for colorimetric estimation of amino acid concentration was essentially that described by Troll and Cannan (1953). The only modification necessary was dilution of the sea water sample with 4 volumes of distilled water which sufficed to eliminate an otherwise troublesome salt error. The procedure was as follows:

1. 0.1 ml of the sea water solution was measured into a 10 ml-Klett test tube and diluted with 0.4 ml of distilled water.
2. 1.0 ml of an 80% solution of phenol in ethanol and 1.0 ml of a KCN-pyridine reagent were added to this sample. This latter reagent consisted of 2.0 ml of 0.01 M KCN diluted to 100 ml with pyridine.
3. The resulting mixture was heated in a boiling water bath. When the temperature of the bath was reached, 0.2 ml of a 5% solution of ninhydrin in ethanol was added and the test tube covered. Boiling was continued for 10 min.
4. After cooling, the sample was diluted to 10 ml with 60% ethanol and the optical density at 540 mμ determined using a Klett colorimeter.

Since ammonia produces a similar color in these circumstances, the phenol and pyridine were kept ammonia-free. At a concentration of 2.0 mM, each sample contained 0.2 μmole of the acid. This produced a deflection of approximately 140 scale units on the colorimeter at 540 mμ. Since the limit of sensitivity of our colorimeteric technique was approximately 0.02 μmole of acid per sample, work with concentrations materially lower than 2.0 mM required concentration of the acid from a larger sample. The following procedure was adopted. Dilutions were prepared from a stock of 2.0 mM glycine to concentrations of 0.1 mM, 0.05 mM, and 0.02 mM. Samples sufficient to provide a calculated concentration of 1.0 μmole/ml were evaporated to dryness on a hot plate. The dried samples were then extracted with 2% HCl in acetone. This extract was dried and the residue taken up in 1ml of distilled water and carried through the estimation procedure previously described. When dilutions were prepared using distilled water, 88% to 95% of the acid could be recovered. However, when sea water was used in preparation of these lower concentrations, the percentage recovery was less and was related to the initial concentration of the sample. Thus only 38% to 50% of the expected concentration could be measured when the sample had an initial concentration of 0.02 mM.

RESULTS

A typical set of observations of the changes in concentration of amino acid may be presented to illustrate the kind of data obtained. A 2-in. specimen of the clam, *Spisula*, was placed in 300 ml of sea water which was 2.0 mM with respect to glycine and which also contained 40 mg/L of tetracycline. Samples for colorimetric determination of the amino acid concentration

TABLE 1. *Uptake of glycine by the clam,* Spisula solidissima

Time (hr)	Colorimeter reading	Decrease in glycine conc.
½	123	–
	124	
	122	
8	98	22%
	95	
	95	
16	15	87%
	16	
	17	
24	2	99%
	0	
	1	

were drawn from the container 30 min after placing the animal in it and subsequently at 8 hr, 16 hr and 24 hr after zero time. Each sample was analyzed in triplicate. Table 1 presents the results of this set of observations.

Comparable results were obtained using artificial sea water as a medium or when streptomycin or penicillin was present. It seems clear that glycine is being removed from the sea water and the occurrence of this in the presence of antibiotics makes bacterial removal improbable. During these observations, a similar container of glycine solution was sampled and analyzed at the same times as the experimental container. No change in concentration was observed. Thus adsorption of the acid on the walls of the container is not a tenable explanation. Shells cleaned by boiling exhibited negligible uptake over a period of 22 hr (1% increase in apparent glycine concentration). Shells from which the animal had been removed leaving fragments of muscle exhibited an increase (30%) in ninhydrin positive material over the same period. Inert materials such as rocks encrusted with coral skeletal material exhibited no uptake. Hence adsorption of glycine by the shell does not appear likely. Finally, the failure of some species to remove amino acids in the survey of animals to be presented provides a further control over various other routes of elimination of the glycine from the sea water. We may then suggest that the animal is acting as an agent in the removal of glycine in these observations.

A survey of marine invertebrates was undertaken to determine the distribution of this ability. No effort was made to restrict attention to those animals which may be classified as filter feeders since it quickly became apparent that this mode of feeding was not a necessary condition for removal of amino acids from solution. In general, two or more sets of observations were carried out for each of the animals listed although there were a few cases where only one set was made. However, the material presented in tabular form gives representative data rather than averages. The time between the initial observation and the terminal sample varied from 16 to 24 hr. Furthermore, neither nitrogen determinations nor oxygen consumption measurements were undertaken in parallel with these observations. For these reasons, one set of observations is not strictly comparable with another and comparison of uptake by the different animals is not justified. Table 2 should then be taken merely as indicating cases in which a significant decrease in amino acid concentration was observed during the period indicated. All of these observations were made in the presence of tetracycline and all were accompanied by parallel analyses of the initial solution with no added animals. Analyses were in triplicate. Initial concentration of glycine was 2.0 mM in all cases. The volume of solution varied from a minimum of 50 ml for *Uca pugnax* to a maximum of 500 ml for *Spisula solidissima.* Weights and volumes were comparable for replicates for a given species.

It is apparent from Table 2 that the capacity for removal of glycine from sea water is rather broadly distributed in the animal kingdom. The outstanding exceptions were the arthropods examined which failed to exhibit this capacity. Shaking the arthropod samples with permutit to remove possible ammonia did not change the observed values at the end of the experimental period. As has been stated, the data presented are representative and not averages. As an

TABLE 2. *Uptake of glycine by marine invertebrates for the period indicated. Initial concentration, 2.0 mM*

Organism	Time (hr)	Decrease in glycine conc. (%)
Porifera		
Microciona prolifera	22	90
Cliona celata	18	38
Cindaria		
Tubularia crocea	18	19
Schizotricha tenella	18	25
Metridium dianthus	21	31
Eloactis producta	18	40
Astrangia danae	21	57
Alcyonium carneum	18	33
Rhynchocoela		
Cerebratulus lacteus	24	58
Ectoprocta		
Bugula turrita	18	70
Annelida		
Nereis virens	17	87
Chaetopterus variopedatus	24	50
Amphtrite ornata	17	33
Sipunculoidea		
Golfingia gouldii	18	36
Mollusca		
Littorina littorea	22	100
Crepidula fornicata	19	22
Mercenaria mercenaria	17	20
Aequipecten irradians	22	37
Mytilus edulis	24	46
Yoldia limatula	23	58
Arthropoda		
Balanus balanoides	23	0
Orchestia agilis	22	1
Limulus polyphemus	22	2*
Palaemonetes vulgaris	16	0
Pagurus longicarpus	23	1
Uca pugnax	22	1*
Echinodermata		
Asterias forbesi	22	97
Henricia sanguinolenta	22	63
Thyone briareus	18	33
Leptosynpata inhaerens	22	62
Hemichordata		
Saccoglossus kowalevskii	18	24
Chordata		
Ciona intestinalis	18	38
Molgula manhattensis	18	35
Amaroucium stellatum	22	22

* Increase

example of variability, 4 *Amphitrite* were observed after 17 hr in a solution of glycine. The decrease in concentration in these 4 cases was 33%, 42%, 45% and 48%. On occasion, an animal would not exhibit this capacity although others of the same species would do so. In particular, *Mytilus*, *Ciona*, *Crepidula* and *Spisula* failed to demonstrate any uptake in some cases.

The isoelectric point of glycine is 6.1 and hence it behaves as an anion at the pH of sea water. To extend our observations, we used a series of amino acids which were chosen to exhibit an isoelectric point on the alkaline and the extreme acid side of sea water and also to provide a variety of chemical structure. Such tests were performed using several different animals whose capacity to take up glycine had been demonstrated. Since *Spisula* was used most extensively, results are presented for this animal in Table 3. *Astrangia*, *Crepidula* and *Metridium* gave comparable results. Thus the removal of these compounds occurs whether they behave as cations or anions at rates comparable to that observed for glycine.

Although our observations strongly suggested an active role of these animals in removal of amino acids from solution, it seemed desirable to attempt to get more direct evidence for this conclusion. A series of samples was taken from the incurrent and the excurrent siphon of *Spisula* and the amino acid concentration of these samples compared. Samples were drawn using a tuberculin syringe. The sample from the excurrent siphon was taken first and that from the incurrent siphon within a minute or so after this. Analyses were in triplicate and were discarded as unsatisfactory if replicates did not agree with 3%. Figure 1 presents the results of all such determinations. The ratio of excurrent to incurrent amino acid concentration is plotted. Each point represents a pair of determinations in triplicate. Six different animals were used. It is apparent that in at least some cases, a clear decrease in amino acid concentra-

TABLE 3. *Uptake of amino acids by the clam,* Spisula

Amino acid	Isoelectric point	Time (hr)	Decrease in conc. (%)
Glutamic acid	3.1	17	29
Tyrosine	5.6	16	34
Methionine	5.7	16	58
Phenylalanine	5.9	16	24
Arginine	10.8	17	20

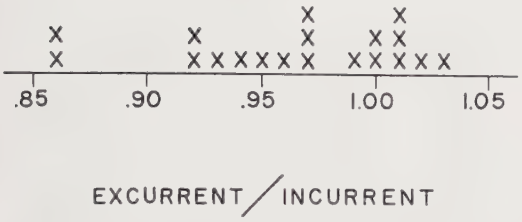

FIG. 1. Ratio of glycine concentrations at the excurrent and incurrent siphons of the clam, *Spisula solidissima*.

tion is occurring during passage of the water through the mantle cavity. It is possible that the cluster of values indicating a slight increase in concentration at the excurrent siphon may be due to excretory products. However, the difference is too small to be defended as significant. The animals were pumping water through the mantle cavity in all cases.

Although it appeared that this capacity for uptake was rather broadly distributed and in at least one case could be presumed to be occurring in the animal on the basis of quite direct evidence, the concentration of amino acid employed was much higher than any concentration of organic material the animal might normally encounter. A solution of glycine at 2.0 mM represents 150 mg of acid per liter of water. This concentration is approximately two orders of magnitude higher than naturally occurring concentrations of organic materials typically reported. Consequently, observations were undertaken using an initial concentration of glycine of 0.02 mM. A set of observations using *Golfingia* as a test organism is presented in Figure 2. Concentration is indicated in colorimeter units after evaporation of the sample as described. Samples were evaporated in duplicate and determined in quadruplicate. The zero point is a distilled water blank and the sea water blank indicated represents samples of sea water carried through the same concentration and extraction procedure. A total of 6 sets of observations of *Golfingia* were consistent with the data presented. *Spisula* and *Thyone* also removed glycine from a 0.02 mM solution.

Although the rather low percentage of

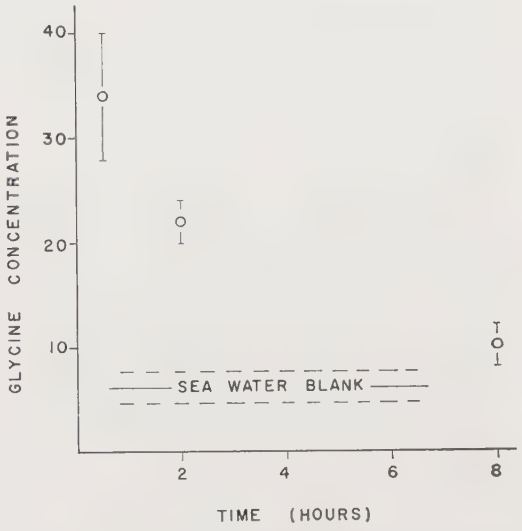

FIG. 2. Uptake of glycine by the spiunculid, *Golfingia gouldii*. Initial concentration of glycine, 0.02 mM. Concentration indicated in colorimeter units.

acid which could be recovered at low concentrations vitiates the quantitative character of these observations, it is apparent that uptake of glycine is occurring at a concentration of 1.5 mg/L. Hence it is not likely that the uptake recorded in the survey of organisms presented earlier is an artifact based on the high concentrations of amino acids employed.

DISCUSSION

It is apparent that the capacity to remove amino acids from solution in sea water is rather broadly distributed among marine animals. The strength of this conclusion depends on the degree to which other pathways for removal of amino acid can be reduced in probability. The disappearance of amino acid in the presence of various antibiotics makes bacterial utilization less probable. Adsorption on the walls of the containers or on the surface of the animals is similarly improbable since no decrease was observed in blank controls nor in any of the arthropods used in these observations. Adsorption on inorganic particles in the water prior to uptake as postulated by Fox, Isaacs, and Corcoran (1952) and subsequent filtration of such particles cannot

be completely eliminated as a possibility on the basis of these observations. It is true that uptake at rates comparable to those reported can be obtained with artificial sea water prepared from distilled water. However, addition of an experimental animal serves as a source of particles. Two additional points suggest that such adsorption is not a critical factor in these observations. First, if such adsorption is assumed, the resulting micelles are apparently not available to such filter-feeding arthropods as *Balanus*. Second, such micelle formation does not provide a mechanism for removal of amino acids by animals which are not filter feeders.

A mechanism of uptake of amino acids involving their retention in the interstices of a net-like filter does not seem attractive, even for those organisms which are ciliary mucoid filter feeders. This would involve an effective pore size for such filters of such dimensions that it is difficult to see how large volumes of water could be processed at low pressures. Quite apart from this, the experiments indicate that uptake occurs in a number of animals in which there is no reason to suspect the existence of a filtering mechanism. Thus *Nereis* removed 87% of the glycine initially present in 17 hr. *Nereis* has been reported to use a mucus net for filtering purposes when pumping water through a tubular burrow (Harley 1950) but under the present experimental conditions, no such device seems available.

The arthropods observed in these experiments did not remove amino acids from solution. Hence, one might speculate on the possible role of mucus since these animals do not secrete it. Adsorption on a mucus sheet has been reported for several invertebrates as a mechanism for the concentration of calcium and strontium (Fretter 1953; Rao and Goldberg 1954). Vanadium is also concentrated in this way by *Ciona* (Goldberg, McBlair and Taylor 1951). Korringa (1952) suggested that this might also apply to organic materials. However, no direct evidence is available for such a role of mucus. It is also possible that the failure of the arthropods observed to remove amino acids may be related to their rather impermeable exoskeleton.

We may consider whether such an uptake of organic material might make any substantial contribution to the food of an organism. According to Jørgensen's (1955) calculations from the literature, 0.05 mg of utilizable organic material must be derived from each liter of sea water processed by a filter-feeding organism in order to provide for maintenance metabolism. Three to four times this yield is necessary to allow for optimum growth. Thus, if the value of 3 to 6 mg/L of dissolved organic material in sea water which is reported in the literature is accepted, a 5% efficiency in removal of this material would yield 0.15 to 0.30 mg organic material per liter of water processed. However, the composition of the organic material in sea water is largely unknown (Vallentyne 1957). It has been argued that the dissolved organic material represents compounds unusually refractory to bacterial oxidation and is consequently of dubious significance to a metazoan. Thus considerations of possible nutritive significance of the uptake reported remain speculative.

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SOME RELATIONS OF ESTUARINE ORGANISMS TO SALINITY

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ABSTRACT

Collectors often ignore salinity while carefully gathering other data with locality records of estuarine organisms, although there has been a great deal of work showing that salinity is a limiting factor to the distribution of many marine organisms, especially as it varies downward, and these limits are often quite sharp. Some recent evidence is reviewed. Hypersalinities are not common in the sea, but where such areas are found on certain coasts, high salinities also limit biotic distributions. The endemic fauna of estuaries is largely sessile. There are a few endemic motile species, chiefly fishes and crustaceans, but the motile fauna is made up mostly of the young of species which spawn offshore in high salinity water and these are sometimes present in vast hordes. Examples are menhaden, mullet, croakers, blue crabs and penaeid shrimp, which all support major fisheries in the United States. Estuaries may be characterized as nursery grounds. They are not refuges for spent races. There are three known types of biological gradients that correspond with salinity gradients. Sessile or only slightly motile marine organisms have optimal salinity ranges for best growth and when the salinities vary away from the optima, either upward or downward, the populations become stunted. A second correlation is between the size of motile organisms and the salinity. In general the smaller and younger ones initially distribute themselves in lower salinity water and migrate towards the sea as they grow larger; thus, the correlation between salinity and size is direct. This correlation is probably dependent upon intrinsic physiological conditions, but more information is needed. The greatest numbers of species of marine plants and animals are found in the photic zone of the shallow sea in full sea water and not in estuaries, as has sometimes been stated. All marine organisms and most estuarine organisms can withstand full sea water, but some of them cannot withstand lowered salinities and thus the species numbers decline with the salinity gradient decline in estuaries. The same thing is noted with the salinity gradient rise in hypersaline areas. The reasons for this situation are not clear, but it shows similarities to the warm to cold decline in numbers of species, away from the tropics, which holds both on land and in the sea. This temperature–species gradient has been explained on the basis that the colder regions of the earth are relatively new and organisms have not yet had time to populate them fully. That explanation is probably not valid, for a parallel situation holds over shorter distances in estuaries, which are not new in terms of the Earth's history.

INTRODUCTION

It is a wearying custom for writers to decry the lack of knowledge in their fields. However, few workers have been concerned with salinity as it is related to marine organisms. People who have had rare opportunities to collect interesting data have often failed to do so. Zoological collectors along our coasts have carefully recorded the county from which their specimens came, with no mention of salinity. Not long ago I read a paper summarizing the records of amphibians and non-marine reptiles in salt water. I am in sympathy with the effort to disabuse zoologists of the idea that amphibians and land reptiles do not venture into saline waters; but I was surprised by the fact that salinity was described mostly in terms of brackish, bay water, salty water, etc. However, the author (Neill 1958) had no other recourse.

SALINITY AS AN ENVIRONMENTAL LIMIT

Biologists should have been overwhelmed long ago with evidence that salinity *per se* is a limiting factor to the distributions of thousands of aquatic organisms. Nevertheless, that great compendium, *Principles of Animal Ecology* (Allee, Emerson, Park, Park and Schmidt 1949), contains the following statement on page 341:

"It seems reasonable to conclude that salinity does not ordinarily function as a limiting factor for animal populations. Sten-

ohaline organisms capable of surviving only narrow changes in salt concentration are usually found in environments in which the salinity is relatively constant. Euryhaline organisms capable of tolerating wider changes in salt concentration are found when considerable variability in salinity is likely. This, of course, follows an obvious point. It can be argued that there is a greater abundance of species and individuals of marine organisms in coastal regions because of the lower salinity existing there. This is a dangerous, and possibly specious, argument *a priori*, however, since the coastal waters may be more favorable in other ways also; e.g., greater food supply. As seems true for pH, it is easy to reach the *ad hoc* conclusion that salinity is an effective factor, but such a conclusion is hard to prove without recourse to experimentation.

"Cowles (1930) supports this point in discussing the ecological distribution of diatoms. 'It is well established from a study of geographical distribution that certain diatoms (oceanic) are characteristic of waters of high salinity, such as that of the open ocean; that others (neritic) are characteristic of waters of lower salinity found along the seacoast and in estuaries; and that still different ones frequent the fresh waters or rivers emptying into the ocean. But, also, it is well known that many of these diatoms are able to stand a large range of salinities and that oceanic as well as neritic diatoms are often found in estuaries where the salinity is very low.' (p. 317)."

That statement contains two errors of fact and cites as support another work which is subject to quite another interpretation than Allee *et al.* put upon it. The diatom populations of bay waters have representatives of oceanic diatoms, just as Cowles stated, but they are not predominant except where offshore water is flowing into the bays; some of them survive for a while because they have some tolerance of lowered salinities and, therefore, are diatomaceous counterparts of the marine fishes and other organisms that sometimes invade bay waters. The bay and oceanic diatom populations are quite different, as Cowles said, and since many of the oceanic diatoms will not withstand lower salinities, we must conclude that salinity is one of the major factors which keep the populations apart.

One of the fathers of marine biology, Stephen Forbes (1859) noted that animals dredged from the shallow bottom of an arm of the sea in Ireland died very quickly at the surface because the water was fresh. This is certainly a rough observation compared to the many more precise measurements made since that time showing that aquatic organisms are rather rigidly delimited by salinity. Allee *et al.* have given examples.

Hypersalinites are not common in the oceanic waters of the world and, therefore, salinity limitations are mostly on the downward side as far as marine organisms are concerned.

Some of the lower limits seem to be quite sharp. Gunter (1956a) and Gunter and Shell (1958) have given several examples. It was shown that young white shrimp, *Penaeus setiferus*, were found in waters with salinities as low as 0.4‰ but they were 140 times more abundant where the water was 0.7 to 0.8‰. Other examples are given in Table 1. Another example is the fish, *Menticirrhus americanus*. Gunter (1945) found the lower salinity limit on the Texas coast to be 14.4‰; Springer and Woodburn found it to be 13.7‰ in the Tampa Bay region (west Florida coast); Gunter and Hall (ms.) found in the St. Lucie Estuary (Atlantic coast of Florida) that is was 12.7‰. The differences are due in part to the different methods of determining salinities.

Segerstråle (1953) has shown that the salinities at four stations in the Gulf of Finland remained at 5.2‰ between 1927 and 1937. In 1949 they rose to about 5.8‰ where they remained until 1952 (the last year reported). The jellyfish, *Aurelia*, and an amphipod, *Calliopius*, extended their range and increased their abundance during this four-year period. Segerstråle pointed out that coincidental extensions of range were noted in other Baltic areas for the cod, sprat, a barnacle, a cockle, a bryozoan and the medusa, *Cyanea*.

TABLE 1. *Some salinity ranges and catches of marine fishes and Crustacea in Grand and White lakes, Louisiana (Gunter and Shell, 1958)*

Species	Salinity ranges	Numbers caught	Number of hauls	Average per haul
Brevoortia patronus	0.18–0.29	10	8	1.3
	0.41–0.93	1,038	15	69.2
	1.14–2.70	1,175	13	90.4
Anchoa mitchilli	0.08	1	3	0.3
	0.10–0.19	283	12	23.6
	0.21–0.93	464	20	23.2
Micropogon undulatus	0.08	2	3	0.7
	0.10–0.93	423	30	14.1
	1.84–4.05	430	12	35.8
Trinectes maculatus	0.08	2	3	0.7
	0.10–0.93	185	30	6.2
	1.84–4.05	64	12	5.3
Callinectes sapidus	0.10–0.73	33	24	1.3
	0.75–0.82	28	4	7.0
	1.14–4.05	58	14	4.1
Penaeus setiferus	0.41–0.47	2	6	0.3
	0.78–0.82	238	5	47.6
	1.84–4.05	326	12	27.2
Penaeus aztecus	0.78–0.82	9	2	4.5
	2.16–2.70	48	7	6.9

During the past 17 years a great many field observations on the lower salinity limits of marine organisms, chiefly fishes of the Gulf coast, have been presented by Gunter (1945, 1950), Reid (1954), Kilby (1955), Gunter and Shell (1958), Springer and Woodburn (1960), and Gunter and Hall (ms.). Simpson and Gunter (1956) and Simmons (1957) have presented upper limits. Gunter (1950), Ladd (1951) and Parker (1959) have shown that the distribution of invertebrates in estuaries and hypersaline lagoons show definite patterns when related to salinity. These observations constitute a large body of information on salinity limits of various organisms, which reinforce one another and throw new light on life history studies. There are many other papers by European workers. Coupled with the relatively few experimental data, these observations constitute powerful evidence that the distributions of estuarine organisms are correlated with salinity. The application of Occam's rule leads to the conclusion that in general limitations on distribution of estuarine organisms are caused by salinity *per se*. There are exceptions, possibly more than we know, and it seems that the above quoted statement of Allee *et al.* was meant to point out the pitfalls of drawing conclusions from individual cases, of which there are examples in the literature. However, they overstated the case and as a general remark the statement is not correct. Because of the wide influence of this great book, the eminent authors should reconsider the matter, if a future edition is printed.

SALINITY GRADIENTS AND BIOLOGICAL GRADIENTS

Salinity and Number of Species

Few workers have run a long series of stations from the fresh water side of a large estuary out to the open sea, counting and recording all species of organisms as they go. Over such a salinity gradient the fauna changes, and there is an increase in the number of species. This increase continues until full sea water is reached. Undiluted sea water near the shore is the optimum for the greatest number of species of animals, definitely not coastal waters of lower salinity, as stated by Allee *et al.* This remark is simply in error. Probably the most extensive data on this subject was given in my own papers (Gunter 1945, 1950). I used trawls, minnow seines, trammel nets and beach seines at 32 stations extending from the mouth of the Aransas River to 5 miles offshore in the open Gulf of Mexico, for a period of 15 months. Thousands of specimens of fishes and invertebrates comprising 160 species were taken. Of these, 122 species were taken at salinities above 30.0‰ and 48 were taken at 5‰ and less.

A similar gradient can be illustrated by any one collecting gear and some evidence has been derived from fouling organisms on oysters (Gunter 1955). Springer and Woodburn (1960) only used minnow seines in a study of the fishes of the Tampa Bay area, but they stated (p. 97), "The number of species, but not necessarily the observed total biomass, decreases as one proceeds away from the Gulf." More evidence on this question will be presented when data

collected on the Caloosahatchee River (the west coast of Florida) are published. This area is a long, narrow body of water with uncomplicated salinity changes, unlike the situation in open bays of broad expanse. It is particularly appropriate for studies of the species–salinity gradient. Day, Millard and Harrison (1952) presented a bar graph showing clearly the decline of species numbers with the fall of salinity in a South African estuary.

The question immediately arises as to why the numbers of species decline as the salinity gradient falls. We do not know, but there are some superficial answers that deserve attention. For one thing, some marine animals can withstand little diminution of salinity from pure sea water. Such species are cut off from any traffic with estuarine waters. Others can undergo moderate changes, and still others can undergo large declines. Such species are said to be euryhaline. This term was coined many years ago by Karl Moebius, who used the term for those organisms which can undergo broad salinity changes. It is not very precise and I attempted (Gunter 1942) to give it a more definite meaning by defining a completely euryhaline organism as one that has been observed living in fresh water and pure sea water by competent observers. Using this thought as a base we can relegate other organisms to relative or partial degrees of euryhalinity.

But animals which can undergo various declines in salinity can also undergo rises in salinity. Thus practically all estuarine organisms can tolerate full sea water, and are often found in it, especially the motile species. Thus the sea contains a great many of the species that live in nearby estuaries, plus a great many that cannot enter the estuaries.

The estuarine fauna, which is typical of estuaries alone, such as the oyster, various clams and worms and crustaceans which must have mud flats or other special environments in which to live are not numerous in species, although the biomass may be great. My impression is that most of the estuarine fauna is sessile or sedentary and the most obvious part is molluscan such as the oyster, the clams, *Rangia*, *Polymesoda*, etc. These are sometimes present in vast abundance; but there is, for instance, one species of oyster in the low salinity bays of the Gulf coast and four in the Gulf, and hundreds of clams and gastropods that are not found in low salinities.

The fresh water fauna does not take over and slowly become progressively more abundant as the salinity falls, thus causing the number of species to rise as the water becomes fresher; it only appears at the lowest salinity ranges. As Pearse (1936) stated, the fauna of estuaries is marine. The estuarine fauna is made up predominantly of marine species or is derived from marine species. There are a few exceptions. Two fresh-water shrimp of the genus, *Macrobrachium*, sometimes enter the bays of the Gulf Coast up to salinities of 17‰, and species of *Palaemonetes* are indigenous along the bay shores. Two species of gizzard shad, Dorosomidae, are commonly found in the bays and sometimes even in the Gulf. They come from fresh water, as do the alligator gars, Lepisosteidae. A few species of the fresh-water *Cyprinodontes* have taken up residence in the bays and are now salt-water fishes. Two species of mouth breeding catfishes are fully marine.

Odum (1953) stated that fresh-water organisms share the estuarine environment with marine organisms up to salinities of 3.5‰ and that it is not correct to say that marine species dominate at these salinities. His idea was evidently an assumption as no data were presented. Gunter and Shell (1958) worked in a large, stable, estuarine area on the Louisiana coast (White and Grand lakes), where salinities ranged from 0.08 to 2.70‰ from September 1951 to June 1953. Samples were collected at 45 stations and 18 of them had salinities of less than 0.3‰ (fresh water). Nineteen marine fishes and crustaceans were caught 5,118 times; 14 fresh and brackish water species yielded 611 specimens. If the three brackish water animals, *Rangia cuneata*, *Palaemonetes pugio* and *Lucania parva* are removed from the list, only 11 fresh-water species and 207 specimens remain. The marine dominance of this low salinity area is about 2 to 1 in

species and about 25 to 1 in numbers. The numbers of species of marine organisms also become less as the salinity increases above that of sea water. Simmons (1957) studied the Laguna Madre of Texas, where the salinities ranged from 27 to 78‰ during his periods of study. He found 72 species of fishes compared to 120 that I found in a more normal area less than 50 miles away. Simmons (p. 190) said that "Certain facts stood out concerning salinity and fauna. The higher the salinity: 1. The fewer the species. 2. The greater the number of individuals of each species available. 3. The larger the average individual of each vertebrate species. 4. The smaller the average individual of many invertebrate species—i.e., blue crabs, barnacles."

With reference also to the Laguna Madre of Texas and other Gulf areas Parker (1959) said: "1. Under conditions involving variable and extreme salinities and temperatures, only a few species of marine invertebrates and individuals of each species may survive. 2. When hydrographic conditions are stable, but salinities and temperatures are in the extreme range, a few tolerant species may become extremely abundant. 3. When physical conditions are stable and within the normal range for marine environments, there will be many species but fewer individuals per species."

Both sets of remarks are extensions in part of the principle that the numbers of species of organisms decrease as the environment varies from the optimum.

Salinity and Size of Organisms

The effect of salinity on the size of organisms is evidenced in two different ways. The first is related to sessile or relatively non-motile organisms. Metcalf (1930) and Andrews (1940) noted that the snail, *Neritina virginia*, grew to greatest size in sea water, but was stunted at lower and higher salinities. Ladd (1951) observed that the brackish water clam, *Rangia cuneata*, grew to greatest size at very low salinities, which are optimum for that species, and specimens from saltier water were smaller. The same observation was made independently by Dr. J. P. E. Morrison (personal communication). Milne (1940) found that sessile plants and animals on buoys became less numerous and smaller towards the lower end of their salinity range. Boettger (1950) noted the same relation of certain mollusks to lower salinity. Other evidence has been presented by Picard and Le Roch (1949) and Segerstråle (1957). In summary, sessile and non-motile organisms have optimum salinities at which they grow best, and when salinity varies away from the optimum either upward or downward, growth is less.

The second correlation of salinity with size is of an entirely different nature and concerns motile organisms. A number of workers on the Gulf coast have demonstrated that a great many of the important marine animals of that area have similar life histories. The adults spawn offshore and the young move back into the estuaries where they grow up in low salinity waters; after a time they return to the sea and the larger adults of many species are found only in the sea. Thus, during the return to salty waters, which comes at a certain stage of their life history, there is a correlation between the salinity of the water and the size of the organism. Weymouth, Lindner and Anderson (1933) were the first to state this salinity/size relationship in clear terms. Gunter (1945, 1950) gave several examples, mostly in tables or merely as comments about larger animals being found in higher salinities, and also presented ten frequency curves illustrating the case.

Correlation of salinity with size of motile animals is only a general situation and there are some obvious exceptions. For example, in such a life history, the very smallest forms are always in the higher salinities and as they drift ashore and enter the estuaries they must be inversely correlated in size with salinity. A few fish, such as *Sciaenops ocellata*, return to the bays after spawning and there are occasionally sizeable populations of larger fish along with the smaller ones.

Lindner and Anderson (1956) have stated that correlations of the size of shrimp with a given salinity do not exist, within certain

wide ranges, and this point is generally correct. The correlation is not with a given salinity, but rather with the gradient as a whole. In other words, the relationship is present whether the salinity range in a given estuary is 5 to 20‰ or 20 to 35‰. This is obvious, or otherwise the life histories and abundancies of the estuarine raised organisms would fluctuate with small annual changes of the general salinity picture.

In all probability the correlation of salinity with size has some physiological basis, for it seems reasonable to assume that animals which each year undergo large cyclic changes of salinity in connection with their life histories have developed physiological adjustments in connection with such changes; and in view of the large osmotic and ionic changes involved, it would seem that physiological adaptations are a necessity. There are some indirect indications that such is the case. I have shown (Gunter 1957) that the marine invaders of fresh water are predominantly the young, and June and Chamberlin (1959) found that young Atlantic menhaden only develop normally in low salinities. The young fish develop abnormally in high salinities and only undergo a change from low to high salinities, without damage, after they have increased in size. Massman, Ladd and Nicholson (1954) have previously given field data showing the affinity of young menhaden for low salinity water. The observations of Lindner and Anderson (1956) led them to believe that shrimp move to the sea as they grow larger as a reaction to salinity which is related to spawning or maturity. I have shown (Gunter 1945) that some fishes begin to move towards the sea in summer when there is no temperature change. For some reason as they grow larger they go into salty water. Marshall and Smith (1930) noted that certain fishes loose kidney glomeruli as they grow and move out into saltier water. Recently, Kawamoto, Kondo and Nishii (1958) have shown that fish can be made to select salt or fresh water by injecting them with thyroid or anti-thyroid extracts.

SOME BIOLOGICAL CHARACTERISTICS OF ESTUARIES

As was stated above, the truly endemic fauna of estuaries is mostly sessile. Certain fishes, such as the cyprinodonts, inhabit the bay shores, although they can withstand astounding salinity changes. Others, such as certain blennies and the clingfish, *Gobiesox,* seem to be tied rather closely to the areas with hard substratum and crevices in which to hide. In the muddy areas of the Gulf coast such an environment is provided only by the oyster reefs. Some species of the genus *Anchoa* are mostly confined to the bays and they are present in great abundance. The preponderant macroorganisms, both in numbers of species and individuals, are mostly motile species which undergo the general type of life history described above. In southern waters these are the mullet, menhaden, croakers, shrimp and crabs. Vast numbers of these animals may be found in estuaries at one time or another and in general the very smallest sizes are found in the lower salinities. The smaller and younger of these species seem to be able to withstand extremely low salinities and this is probably the reason why the chief invaders of fresh water are the small or young. It should be noted that the definition euryhaline applies to species as a whole and not to individuals.

Estuaries are predominantly nursery grounds. In this respect it is significant that the greatest regions of crab, croaker and menhaden production of the United States coast are Louisiana and the Chesapeake Bay area. These regions are also similar in supporting the greatest oyster production, and Louisiana has the greatest white shrimp production.

Annandale (1922, p. 154) said " . . . fresh and brackish water have proved a last refuge for many marine animals whose race in the sea was nearly done." Therefore, estuaries have been called refuges for spent races. That is a wrong view. Estuarine organisms can undergo wider changes of physical and chemical characteristics than the ordinary denizens of either fresh water or sea water, and they show many char-

acteristics of individual and species vigor. Estuaries are better characterized as nurseries. However, there is something to the idea of estuaries as refuges and this is almost implied in speaking of them as nursery grounds. It has been said that oysters invaded estuaries because of enemies. We know that oysters in high salinity waters suffer depredations from more enemies and parasites than those in low salinity waters (Gunter 1955) and the fact is taken into consideration by oyster growers. Furthermore, there is no known reason why an oyster cannot get along well physiologically in full sea water. The same idea has been advanced concerning estuaries as nursery grounds for young shrimp (Gunter 1956b).

CLASSIFICATION OF SALINE WATERS

Several classifications of saline waters have been proposed. These have been summarized by Segerstråle (1959) and I shall not discuss them here. The only broadly different type of classification offered in recent years is that of Odum (1953) in which he gave the classification of all waters in ranges of chloride in parts per thousand. These were 0.0019–0.019, 0.019–0.19, etc., up to 19.0‰. The range thus runs from rain water to sea water but unfortunately, the categories bear no particular relationship to the fauna. In the first place, the invasion of fresh water by marine organisms is not dependent on the amount of chloride in the water; the important factor is the presence of calcium in the fresh waters. Physiologists immediately recognize the importance of this factor, but strangely it has been emphasized only by Breder (1933). Odum's third range covers from fresh water up to a salinity of about 3.5‰, and so corresponds somewhat to the oligohaline classification of most workers. However, the next one jumps from a salinity of 3.5‰ to full sea water, and does not have any useful correlation with the distribution of marine organisms.

The so-called oligohaline zone of most classifications of sea water and Odum's corresponding range do not go quite high enough. My observations have been that the various fresh-water organisms that invade the sea generally go into salinities somewhat higher than 3.5‰. The oligohaline zone purports to begin in fresh water which is called 0.5‰. A salinity of 0.3 to 0.5‰ is equivalent in total salt to hard fresh water. A salinity of about 0.1‰ is equivalent to soft fresh water. Strangely enough, you can have sea water diluted with fresh water, with the chemical components and proportions of sea water at a lower salinity than 0.5‰ (hard fresh water). We have found such cases in Florida and this is the type of water in Grand Lake and White Lake in Louisiana. Therefore, we may inquire what is the actual limit between low salinity sea water and fresh water? It is not at any given salinity, but it varies with the types of drainage and the real limit comes about where the proportion of total salts to the chloride begins to change and become greater than 1.8.

Classification of saline waters has generally been in terms of fresh water, oligohaline, mesohaline, metahaline (sea water) and hyperhaline, but a number of terms have been proposed. A great many words have been written and spoken, attempting to correlate these divisions with the biota. It would appear that actual counts of species of organisms over salinity gradients when correlated with the salinities would give an objective answer to this question.

THE SPECIES GRADIENT QUESTION

The decrease of species over the falling temperature gradient is a well-known phenomenon. Forbes (1859) noted it in the seas of the world and intimated that the numbers of species decreased because it simply got too cold along the gradient for some organisms. We seem to have progressed no farther for Dobzhansky (1950) came to the same conclusion in a discussion of the species–temperature gradient on land. This strikes me as a circular argument and no answer.

In any case the species gradient situation can be much more easily studied in estuaries, where it is correlated with a relatively short salinity gradient in terms of miles. This question is related to many ecological

and physiological questions, as well as to problems of evolution, and I recommend it to vigorous, imaginative, young workers. Prosecution of this work should shed light over a broad field. For instance, the idea has been advanced that there are not many species in colder climes because the cold poles are relatively recent and the invading organisms of colder regions have not had time to diversify. The idea sounds plausible until we examine it in the light of what is known of estuaries, where we also have reduction in numbers of species. I do not think that anyone would theorize that estuaries are recent in terms of Earth history.

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STRUCTURE AND ENERGY FLOW OF A MUSSEL POPULATION IN A GEORGIA SALT MARSH[1]

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ABSTRACT

Modiolus demissus Dillwyn was studied near Sapelo Island, Georgia. The size distribution of the mussels was bimodal with maximum numbers in the dry weight (body) classes 0–24 mg and 400–599 mg. The total weight of mussels smaller than 200 mg was less than 2% of the total population weight. Random samples gave an estimated population density of 7.8/m^2 for the entire inhabited marsh; the estimated organic biomass was 11.5 gm/m^2, one third of which was body and two thirds was shell conchiolin. The population was most dense near the heads of small creeks, averaging 32 mussels/m^2. The mussels, however, were not only more abundant in some types of marsh than in others, they were also clumped within areas of uniform marsh. Shell growth occurred throughout the year, especially in small and medium-sized individuals, but it was more rapid during the warm months. The height-to-weight ratio changed with the seasons, the mussels being heaviest prior to spawning. Growth and mortality of mussels in flower pots in the marsh indicated a net annual population growth of 445 mg/m^2 (dry body weight) and a net annual mortality loss of 1200 mg/m^2. Allowance for bias would place the steady state growth–mortality rate necessary to just maintain the population somewhere between these two estimates. Population growth and gamete production accounted for an energy flux of about 13.9 and 2.8 kg-cal/m^2/yr, respectively. Respiration by the population accounted for approximately 39 kg-cal/m^2/yr, over two thirds of which took place in air during ebb tide. Total assimilation amounted to 56 kg-cal/m^2/yr.

INTRODUCTION

The present study was part of a program of estuarine ecosystem investigations carried on at the University of Georgia Marine Insitute at Sapelo Island. The ribbed mussel population was chosen because it constitutes an important fraction of the total animal biomass of the marsh and could, therefore, be expected to influence the energy and nutrient budgets of the ecosystem. Furthermore, it is the only important pelecypod population on the marsh flats above the creeks but below dry land west of Sapelo Island (Fig. 1). Teal (1958) described the morphology and plant cover of this salt marsh area and illustrated a transect through the most important marsh types. Ragotzkie and Bryson (1955) studied the hydrography of the Duplin River, the watercourse which floods and drains most of the study area during each tidal cycle.

The ecology of the ribbed mussel, *Modiolus demissus* Dillwyn, has never been thoroughly investigated even though this species is abundant and widespread. According to Abbott (1954) it is found from the Gulf of St. Lawrence to Yucatan. McDougall (1943) reported this mussel in the reed beds near Beaufort Harbor, North Carolina and at intertidal levels on wharf-piles. Young individuals appeared in the last week of August and continued to set through October. He reported maximum setting 6 in. below the low water mark with setting as high as 24 in. above low water. Sullivan (1948) found the larvae of *M. demissus* present in the waters of Malpeque Bay, Prince Edward Island, from mid-July through August, during which period the water temperature changed from approximately 22° to 24° to 18°C. She also described and illustrated the larva and spat. Chestnut (1951) gave a brief account of the economic uses of ribbed mussels and mentioned their characteristic clumped distribution.

Near Sapelo Island *Modiolus* is found at upper intertidal levels in the salt marsh. Each mussel is attached by its byssus to

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FIG. 1. Study areas in salt marsh west of Sapelo Island. Squares are population density sampling areas; rectangles are transects with pots; Location A is the weight-class distribution sampling area; Location B is Marsh Landing.

plant roots or to other mussels. Each has its posterior margin projecting above the mud to permit feeding. Very small mussels are capable of crawling and climbing; in laboratory trays they tend to group together. Small mussels in nature are frequently attached to the posterior margins of the valves of larger individuals. A large mussel can release itself from its byssus, but it is heavy relative to the strength of its foot and, therefore, its power of movement is limited. In the marsh mussels are usually found in clumps. These aggregations are especially noticeable where the vegetation is short because they form mounds several centimeters high, and the grass which grows above the clump is somewhat taller than average. Since large mussels have very limited motility, the clumps probably result from attraction of small mussels to ones already established and, perhaps, from enhanced growth or lessened mortality in the group. Mussels are well adapted to enduring exposure during low tide. They are largely protected from the sun by being embedded in the mud, and Kanwisher (1955) found that they could survive several days' exposure to –22°C, at which temperature 71% of the body water was frozen.[3]

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METHODS

Frequency Distribution of Weight Classes

Every two months between November 1, 1958 and May 1, 1959, mussel colonies were analyzed for the size-class distribution of the individuals in the population. Entire colonies were dug up from an area of marsh (Fig. 1, Location A) and transported to the laboratory where the mussels were carefully separated from the mud and roots of the marsh grass, *Spartina alterniflora*. When all mussels had been recovered, the shell height of each was measured. Height was found easier to measure than length and showed nearly the same statistical variation. The shell height measurements were tabulated in one millimeter increments, the mean body weight per mussel of each height class was calculated from the current height-to-weight regression,

[3] His *Modiolus modiolus* was actually *M. demissus* (personal communication).

then this mean body weight was multiplied by the number of mussels in the class to get the total weight of body tissue per height class. The mussels were then grouped into the weight classes: 0–24, 25–49, 50–99, 100–199, 200–299, 400–599, 600–799, 800–999 and 1000+ mg.

Population Density and Distribution

Twenty-five sampling areas, each 14,400 m^2, were selected at random from a 1,140 hectare tract of salt marsh just west of Sapelo Island (Fig. 1, small squares). A grid was prepared as an overlay to a map of the area. The random selection of sampling areas was accomplished by finding coordinates of the grid which corresponded to numbers taken from a table of random numbers. Within each sampling area ten 0.2-m^2 quadrats were selected by pacing off coordinates which had also been taken from a table of random numbers. After recording the type of marsh wherein the quadrat fell, the vegetation within the quadrat was clipped down to the mud surface and all the mussels were dug up. If a quadrat fell on a type of habitat completely unsuitable for *Modiolus*, such as in a small creek or on land above the high tide level, it was rejected and replaced by another randomly selected quadrat. Thus 250 samples were eventually obtained from potential *Modiolus* habitat. The mussels in each sample were brought to the laboratory to be measured and weighed.

The heights of the various types of marsh above the datum plane (approximate mean low water; Ragotzkie and Bryson 1955) of the tide staff at Marsh Landing (Fig. 1, Location B) were measured by comparing the maximum depth of water over the marsh at high tide with the maximum height of the same tide as recorded at Marsh Landing. Some measurements of water depth were made on-the-spot during the short period of high water; others were made using devices designed by Dr. J. M. Teal. Each device consisted of a stake over the top of which passed a string bearing a float on one end and a counterweight on the other. These stakes were erected in the marsh before high tide so that the rising water would lift the float; the counterweight would prevent the float from falling down when the tide went down. The position of the float gave a record of the maximum water depth at that point in the marsh. The number and duration of covering tides were determined by measuring the number and duration of tides which exceeded 190, 220 and 250-cm elevation on a group of randomly selected weekly tide records. These tide records had been produced by a recording tide gauge at Marsh Landing during the previous two years.

Recruitment, Growth and Mortality Rates

Seven areas representative of the entire marsh were selected and in each area a transect 18 to 100 m long was established (Fig. 1, small rectangles). Between 5 and 15 small flowerpots were set into the marsh mud along each transect so that the mouth of the pot was flush with the level of the surrounding mud. A numbered stake was driven beside each pot as a marker. Three or more, usually five, mussels were measured for shell height and placed in each pot along with mud from the adjoining marsh. The mussels were oriented in their normal position with the siphon end upward, and the ventral side was placed as near as possible to the inside of the pot to allow the mussel to construct a new byssal support easily. The population density per unit area of mussels in the pots was about the same as that of an average mussel colony.

Every two months the pots were visited and the mussels were detached by cutting the byssal threads. The mussels were washed in a bucket of sea water to remove excess mud, measured and then returned to the same pot as before. If a mussel died or disappeared, a measured replacement was put in that pot. On those occasions when natural recruitment of young occurred, either by direct settling out from the plankton or by migration into the pot, these recruits were measured and returned to the pot with their neighbors. Since the smallest mussels (0–24 mg) frequently wander, it was sometimes impossible to be certain that

a particular small mussel in a pot was the same one which had been measured previously. When any doubt arose, that mussel was treated as a recruit and the previous one was considered lost by mortality.

The recruitment, growth and mortality rates of the population were computed bimonthly as the change in body weight per square meter of average marsh. An estimate of dry body weight of every mussel in the pots was obtained by use of the current height-to-weight regression. Each mussel was then assigned to one of 9 weight classes according to its weight at the beginning of the two-month period. The total weight of mussels in each size class at the end of a two-month period was divided by the weight at the beginning to obtain the relative, or percentage, increase or decrease.

Bimonthly gains in biomass per square meter due to recruitment was computed by the formula:

$$\text{Recruitment} = N_r W \frac{N_m}{N_p}$$

where N_r is the number of recruits entering the pots during the two months, W is their mean weight, N_m is the average number of mussels per square meter of the entire marsh, and N_p is the total number of mussels in pots.

The growth rate for each two-month period for each weight class was calculated as:

$$\text{Growth} = B\,G\left(100 - \frac{M}{2}\right)$$

where B is the biomass of the weight class per square meter of average marsh, G is the relative growth rate, and M is the relative mortality rate. It will be shown later that growth rate of mussels was significantly less at high elevations than at low elevations in the marsh, but this difference in growth rate was small. Since, in addition, distribution of the pots was similar to that of the natural population, the mean growth rate calculated from the pot experiment was considered to be the same as that of the population.

Deaths and disappearances were both included in the mortality estimate. This loss was calculated as:

$$\text{Mortality} = B\,M\left(100 + \frac{G}{2}\right)$$

where B, M and G are the same as in the growth equation.

The rate of byssus production was measured in the marsh by placing mussels in 250-ml beakers and harvesting the byssal threads every two to three months. The beakers were filled with sieved mud at the beginning of each period so that the mussels found no byssal attachment surface except the inside of the beaker. The many *Spartina* rootlets otherwise present in the byssus were thereby eliminated, and the dry weight of the byssus could be accurately determined.

Oxygen Metabolism

Oxygen consumption by mussels of different sizes at various temperatures was measured both while mussels were submerged in sea water and while they were in air. The mussels were acclimated to each experimental temperature for at least 12 hr, and experiments were run in a constant temperature bath. Respiratory rates of submerged mussels were obtained in March by measuring the decrease in dissolved oxygen by the Winkler method. Respiration in air was determined in July with a Scholander-type volumetric apparatus (Wennesland 1949). The regressions of respiratory rate on body weight at various temperatures were combined with knowledge of the frequency distribution of size classes, monthly mean temperatures, and duration of marsh flooding to estimate total annual oxygen consumption by the population. A factor of 4.86 kg-cal/ml O_2 (assuming R. Q. = 0.85) was used to convert oxygen consumption to the caloric equivalent. Caloric content of body tissues was measured in a Parr adiabatic oxygen bomb calorimeter.

Statistical Treatment

The statistical handling of data was based primarily upon Snedecor (1946). Unless otherwise stated, mean values are followed

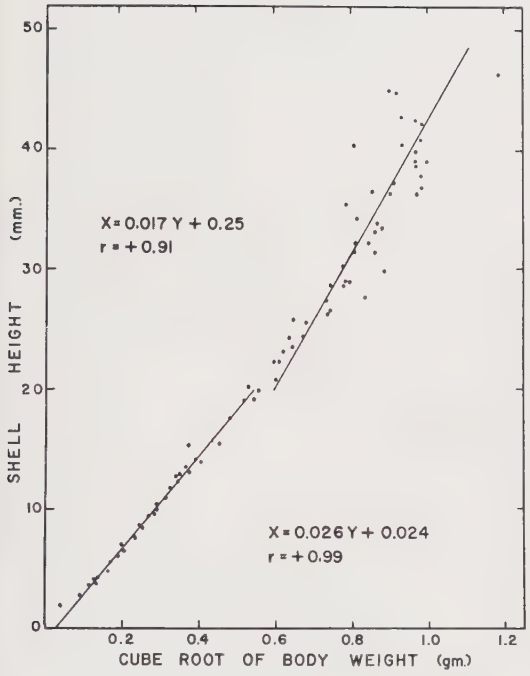

FIG. 2. Height-to-weight regression for *Modiolus* in late April, 1959.

by the standard deviations in parentheses. Very small samples ($n < 7$) were assumed to be normally distributed, small samples ($n = 7$–30) were tested graphically by the rankit method (Bliss and Calhoun 1954) and large samples ($n > 30$) were tested for skewness and kurtosis by the g_1 and g_2 statistics. Statistical treatment of non-normal distributions will be discussed with the appropriate data.

RESULTS

Height-to-Weight Ratio

There is a good correlation between the height of a mussel shell and the cube root of the shell-free, dry body weight. It was possible, therefore, without killing an animal, to make an accurate estimate of its body weight by use of a height-to-weight regression. Figure 2 is an example of such a regression. Although the inflection point on the curve appears to be at about a shell height of 25 mm, the two lines were divided at 20 mm since this is the approximate size at which mussels reach sexual maturity. The very straight line up to 20 mm on this cube-root transformation indicates isometric growth to this size. Mussels above the inflection region have less body in proportion to shell than those below it. To compare the present data with those of other workers, shell length is 2.5 times shell height. The wet weight of the body fraction averages 6.7 ($s = 0.8$) times the dry weight. The term "body weight" always refers to shell-free, dry body weight in this paper.

TABLE 1. *Regression formulae for computing dry weight of the body from measurements of shell height of* Modiolous demissus. *X is the cube root of the weight in grams; Y is the shell height in millimeters. The division between small and large mussels is at 20.0 mm shell height*

Date	Small mussels	Large mussels
June 9, 1958	$X = .027Y + .027$	$X = .018Y + .31$
September 2, 1958	$.026Y + .026$	$.019Y + .18$
November 1, 1958	$.026Y + .032$	$.020Y + .19$
January 4, 1959	$.025Y + .038$	$.019Y + .21$
March 2, 1959	$.025Y + .042$	$.018Y + .23$
April 28, 1959	$.026Y + .024$	$.017Y + .25$
July 14, 1959	$.025Y + .038$	$.021Y + .17$

The regression formulae for calculating body weights from shell heights were not the same throughout the year (Table 1). Except for the small mussels on September 2, all regressions are based on 40 to 90 individuals collected from a representative area of marsh (Fig. 1, Location A) on the given dates. The formula for small mussels in September is an interpolation between samples obtained on June 9 and September 29. Analysis of variance of the regression formulae (Bliss and Calhoun 1954) showed significant seasonal changes in the slopes of the bimonthly regressions both for small and large mussels ($F = 2\%$ and 4%, respectively). Furthermore, it showed that the positions of the regression lines changed very significantly, especially in the case of mature mussels as they lose weight during the spawning season. Since the regressions for *Modiolus* change significantly during the year, the appropriate regression was used each time body weights were calculated.

TABLE 2. *Frequency distribution of weight classes of the* Modiolus *population*

Weight Class (mg)	Mean per cent of total number	Mean per cent of total weight	Mean weight for marsh (mg/m²)
0–24	18.4	0.24	10
25–49	2.5	0.20	8
50–99	3.4	0.55	22
100–199	2.8	0.85	34
0–199	27.1	1.8	74
200–399	13.1	9.6	388
400–599	29.8	34.7	1,425
600–799	19.2	30.6	1,257
800–999	8.0	16.3	670
1000+	2.7	7.0	293
Total	100	100	4,107

Frequency Distribution of Weight Classes

Comparison of the frequency distributions obtained bimonthly showed no marked seasonal changes between November and May, so all the data have been combined in Table 2. Although mussels less than 200 mg contribute 27% of the total number of mussels, they form only 1.8% of the total population biomass.

The number of individuals in each weight class of the April 28 population weight-distribution sample is similar to the number present in the pots when visited between April 29 and May 1, 1959 (Fig. 3). These particular examples were chosen because they were large and comparable in size at that time. Almost all of the mussels in the first weight class in the pots were natural recruits. The similarity in the shape of the curves indicates that the pots exerted no pronounced size-dependent effect upon recruitment and mortality. The bimodal shape of the curve is indicative of high mortality in the small mussels (see also Table 6). Since so few mussels are present in the 25 to 199-mg size classes, the large number of mature individuals in the population indicates a lower mortality rate in mussels larger than about 200 mg. Actual rates of recruitment and mortality during the period of the investigation will be discussed in a later section.

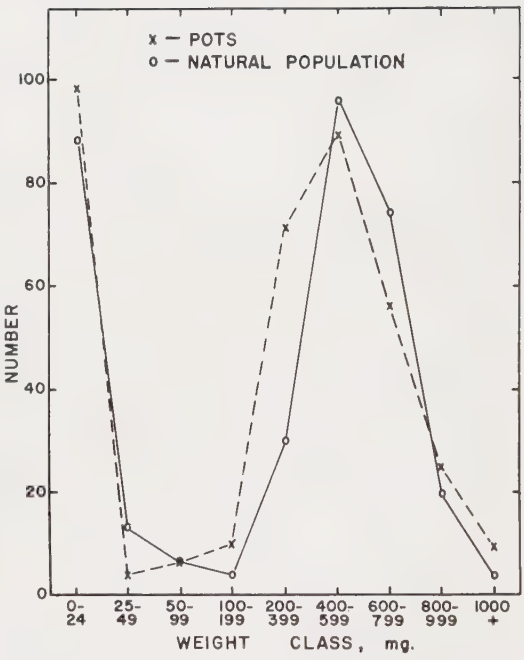

FIG. 3. Frequency distribution of weight classes of *Modiolus* in pots compared with that of the natural population. The total number of mussels in pots was 371; the total number in the sample from the natural population was 335.

Population Density and Distribution

The population density of mussels varied markedly in various types of salt marsh (Table 3). The percentage of the whole marsh constituted by each type was determined by the proportion of randomly selected quadrats which fell in that type. The most striking fact is that 46% of the total population weight occurs in Tall-*Spartina*-Creek-Head marsh even though this type accounts for only 6% of the total marsh area. Medium-*Spartina*-Levee marsh has a density (gm/m²) only one-seventh that of Tall-*Spartina*-Creek-Head marsh, but the levee marsh is 3.5 times as extensive and therefore contributes the second largest percentage of the total weight. In each marsh type, the variance is larger than the mean (Table 3, columns 4 and 5) indicating nonrandom distribution; this will be discussed below.

The range in elevation of marsh types 3 to 9 and the range in elevation of the mussel

TABLE 3. *Population distribution and density of* Modiolus demissus

Marsh type No.	Description	% of total marsh	Number/0.2 m² Mean	Number/0.2 m² Variance	Density/m² Number	Density/m² Wt (g)	% of total population Number	% of total population Weight
1	Muddy creek banks	4	0		0	0	0	0
2	Tall *Spartina*,* edge	9	0		0	0	0	0
3	Medium *Spartina*, levee	21	1.14	12.32	5.7	4.35	17	22
4	Short *Spartina*, low elevation	16	1.24	9.29	6.2	3.0	15	12
5	Short *Spartina*, high elevation	20	0.08	0.11	0.4	0.15	1	1
6	*Salicornia* spp.–*Distichlis spicata*	9	0.23	0.37	1.1	0.40	2	1
7	*Juncus roemerianus*	4	2.64	26.4	13	6.15	9	7
8	Tall *Spartina*, creek heads	6	10.4	146	52	31.6	47	46
9	Medium *Spartina*, medium elevation	11	1.11	5.72	5.6	4.55	9	12
	Total	100					100	100
	Mean		1.33		6.66	4.11		

* Tall *Spartina*, 1–3 m; Medium *Spartina*, 0.5–10 m; Short *Spartina*, less than 0.5 m.

population are shown in Figure 4. Since *Modiolus* seldom occurs in the lower third of the range of type 8, nor in the upper two-thirds of type 3, the range of the mussel population is approximately from 200–240 cm above mean low water (MLW). The center of population biomass occurs very near 220 cm and the maximum density is probably near that level. From the percent coverage graph, one may see that a mussel at 220 cm elevation will be covered by 77% of the flood tides, or about 18% of the time. The mean water depths of those tides which inundate elevations 190, 220 and 250 cm above MLW are 37, 21 and 10 cm, respectively.

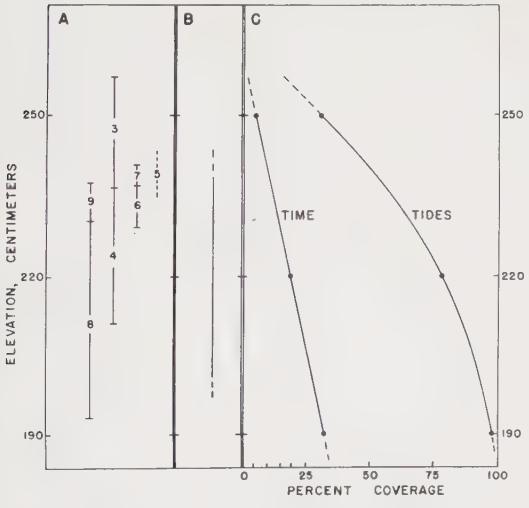

FIG. 4. A. Range in elevation above mean low water of marsh types 3–9. B. Range in elevation of the mussel population. C. Curves showing percentage of time inundated, and percentage of high tides which inundate at various marsh elevations.

The clumped distribution in the salt marsh is a striking feature of the *Modiolus* population. The observed frequencies of mussels in the population density samples from marsh types 3, 4, 8, 9 and 3–9 inclusive agree with the negative binomial distribution ($P = 0.20$, 0.10, 0.09, 0.50 and 0.85, respectively) calculated by the method of Bliss and Calhoun (1954). The clumps of mussels are both larger and more numerous in the densely populated marsh types. In the creek heads, the clumps may be more than a meter in diameter or may extend for many meters parallel to the creek. The greater population density at low and medium elevations (marsh types 4, 8 and 9, Table 2) seems reasonable in view of the greater feeding time available to mussels there (Fig. 4).

The growth rates of mussels in the lower marsh (types 4 and 8, Fig. 3) were compared with those in the higher marsh (types 3, 6 and 9) in the size classes 200–399, 400–599, 600–799, 800–999 during May–June, July–August and September–October, the periods of greatest physiological activity. In 10 pairs the sample size was large enough to make valid comparisons. The growth in lower marsh types was more rapid in every case. Although the mean difference in growth rate was only 2.5%, this difference is probably significant; "Stu-

TABLE 4. *Recruitment rate of* Modiolus *in pots during two-month periods*

	January February	March April	May June	July August	September October	November December
Number of recruits/ established mussel	.050	.24	.18	.028	.078	.045
Mean body weight of recruits (mg)	1.5	.49	1.1	3.0	3.0	1.1
Total body weight of recruits/established mussel (mg)	.074	.12	.21	.086	.23	.051

dent's" t tables show: in one case $P = 0.65$; in one case $P = 0.10$; in 8 cases $P < 0.05$. The growth rates at the extremes of the range in elevation of the mussels probably differ by more than 2.5% since mussels at lower elevations have more time to feed each day and are exposed to stronger water currents, and therefore have more food available. Indeed, Field (1922), Mossop (1923), and Newcombe (1935) found that *Mytilus edulis* grew faster at lower elevations and that this factor was accentuated by favorable water currents.

The total number of mussels collected was 333 from a total sample area of 50 m^2. This gave an average population density of 6.66 mussels/m^2 for the entire marsh. Since the mud was not sieved when these samples were taken, mussels smaller than about 25 mg were not taken quantitatively; only 13 of the 333 mussels found were smaller than 25 mg. The population weight-distribution study (Table 2) showed that mussels smaller than 25 mg constitute 18% of the total number, which is 22% of the number larger than 25 mg. This correction factor gave an expected total of 391 and a corrected average of 7.82/m^2. It will be assumed hereafter that 6.40/m^2 is the mean density of large, established mussels and 7.82/m^2 is the mean density of all the mussels present. The mean density in terms of body weight was 4.11 g/m^2 (Tables 2 and 3). Since mussels less than 25 mg represent only 0.24% of the total weight of the population, this estimate of biomass per unit area is practically unaffected by any incompleteness in recovery of the smallest mussels.

The shell-to-body weight ratio changes as *Modiolus* grows (Fig. 2), but the average ratio for this population was 17 to 1. The weight of whole shell, therefore, should be 17 times as great as the weight of the body fraction, or about 70 g/m^2. The organic conchiolin, measured as loss on ignition, of 20 mussel shells over the height range 9.6-45.8 mm was 10.6 ($s = 0.08$) per cent of the whole dry shell weight, or about 7.4 g/m^2. The uniformity of organic content in shell is remarkable. The total dry organic standing crop equals 11.5 g/m^2, of which almost two-thirds is in the shell.

Recruitment, Growth and Mortality Rates

The largest number of recruits in pots (Table 4) was found in March–April. The larger average size of the new arrivals in September–October, however, caused this latter period to contribute more biomass.

The occurrence of very small mussels throughout the year was not expected since ripe adults are found only in late summer. The explanation probably lies in a highly variable growth rate which, in turn, depends upon the conditions where each individual first settles. Mossop (1923), for example, found that specimens of *M. edulis* from the same bed at the end of the first growing season had shell lengths from 5 to 22 mm.

The shell growth rate of *Modiolus* is highest in spring and summer, and it is more rapid in small mussels than in large ones. Figure 5 shows the course of shell growth of 12 mussels in Short-*Spartina*-Low marsh (solid lines) and 14 mussels in Tall-*Spartina*-Creek-Head marsh (broken lines). These mussels were selected for presentation simply on the basis that they, as individuals, lived at least 6 months and

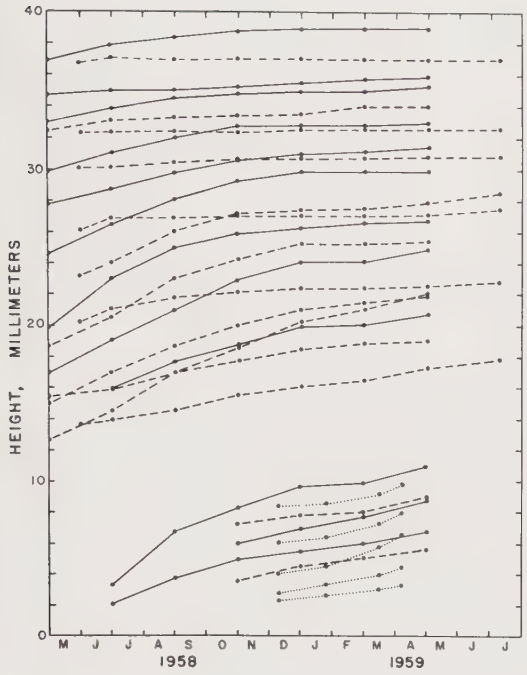

FIG. 5. Seasonal increments in shell height of mussels in Short-*Spartina*-Low marsh (solid lines), Tall-*Spartina*-Creek-Head marsh (broken lines) and in the laboratory (dotted lines).

had representative, although not necessarily average, growth increments. Five mussels (dotted lines, Fig. 5) which were kept in running sea water in the laboratory from December through March compared well with the smaller animals in the field during the same period. Equal slope, of course, represents greater relative growth for a small mussel than for a large one. From the slopes of the lines it appears that *Modiolus* takes at least two years to reach maturity (shell height approximately 20 mm).

The changes in body weight, or relative bimonthly growth rates, in Table 5 are negative during parts of the year in the larger size-classes but the net change for the year in each class is positive. The largest losses in weight during July–August were associated with the spawning period which extends through those months and into September. The mature mussels lost weight even though shell growth did not stop during the spawning period (Fig. 5). May and June are the months of most rapid growth, the small mussels growing relatively faster than the large ones.

The relative bimonthly mortality rates (Table 6) show clearly the effects of age and season. The smallest animals had the highest mortality rates throughout the year, a common phenomenon in nature. Summer and fall months were the periods of greatest loss. There were almost no losses in winter. It must be remembered that migration of the mussels into or out of the pots cannot be distinguished from recruitment from the plankton or from mortality, respectively. Since the pots had about the same density of mussels as the average colony, however, the migrations into and out of the pots should have been similar to the rates for natural colonies in the marsh.

Table 7 presents the changes in weight of mussel body, shell and byssus per square meter of marsh. The recruitment values are all positive, but they contribute little to the total changes. The values for growth or mortality are the sums of the products of

TABLE 5. *Bimonthly percentage change in body weight of nine weight-classes of* Modiolus

Weight class, (mg)	January February	March April	May June	July August	September October	November December
0–24	20.2	11.4	94	102	89	31
25–49	2.9	4.8	49	17	25*	2.7
50–99	2.2	7.3	38	21	19	1.0
100–199	0.07	6.4	39	14	18	4.3
200–399	–4.0	2.2	22	–20	14	6.8
400–599	–6.4	–1.2	21	–19	11	3.1
600–799	–7.8	–2.3	22	–17	10	1.6
800–999	–8.6	–3.1	22	–14	10	0.63
1000+	–9.5	–3.7	20	–12	9	0.02

* Estimated

TABLE 6. *Mortality rate of* Modiolus *in pots, expressed as percentage which died during each two-month period*

Weight class (mg)	January February	March April	May June	July August	September October	November December
0–24	3.2	38	80	54	81	14
25–49	0	0	50	50	67	0
50–99	0	0	33	50	17	0
100–199	0	0	0	27	19	0
200–399	0	0	6.7	14	17	1.8
400–599	0	0	4.3	16	7.4	1.4
600–799	0	0	0	12	16	0
800–999	0	7.4	0	4.6	0	0
1000+	0	0	0	6.9	27	5.9

the values in the last column of Table 1 (biomass/m^2) times the values in Table 5 or 6 (relative growth or mortality). The growth estimate was then corrected for mortality during the period by multiplying by the factor $\left(100 - \frac{\%\ \text{mortality}}{2}\right)$; the mortality estimate was corrected by the factor $\left(100 - \frac{\%\ \text{growth}}{2}\right)$. The use of these corrections produces values of net change in population weight which are in good agreement with net values calculated using the formula from Ricker (1958):

$$W_t = W_o e^{(g-i)t}$$

where g and i are the instantaneous growth and mortality rates, respectively, and W_o and W_t are initial and final population weights. The amount of matter lost by byssal growth amounts to 320 mg/m^2/yr. The change in biomass of the liquor fraction was not estimated but it would be small compared to the body fraction. The changes in shell mass were computed from the changes in the body fraction. The shells of recruits weighed about 11 times as much as the dry bodies, so the recruit shells contributed 66 mg/m^2/yr (Table 7). Most of the growth and mortality, on a weight basis, occurred in mussels between 200 and 999 mg body weight. The annual changes in shell mass of large mussels were 17 times the changes in body biomass due to growth and mortality, or 7,550 and –20,400 mg/m^2, respectively.

The net annual population growth during the period of investigation was only slightly more than one-third the total annual mortality loss (Table 7). It is not possible to determine from these data whether the nat-

TABLE 7. *Changes in the mussel population weight*
Values are mg/m^2

	Recruitment	Growth	Mortality	Net
BODY				
January–February	0.58	–287	–0.34	–287
March–April	0.91	–63	–53	–115
May–June	1.6	889	–121	770
July–August	0.68	–620	–472	–1,091
September–October	1.8	427	–508	–79
November–December	0.40	99	–46	53
Per Year	6.0	445	–1,200	–749
SHELL				
Per Year	66	7,550	–20,400	–12,800
BYSSUS				
Per Year		320		320

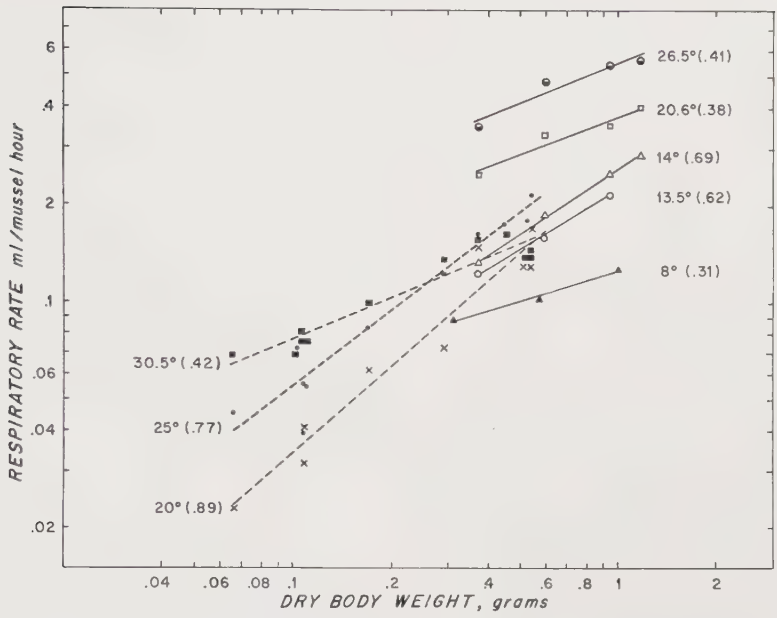

FIG. 6. Logarithmic plot of oxygen consumption in water (solid lines) and air (dashed lines) against body weight of *Modiolus*. The temperatures are centigrade. The figures in parentheses are b, the slopes of the least square regressions.

ural population biomass really declined, or whether the growth or mortality estimates, or both, are biased. The disturbance associated with the bimonthly measurements, especially the cutting of the byssal threads in order to remove the mussels from the pots, may have significantly increased predation losses. In addition, energy expended in producing a new byssus would not be available for growth. It is felt, therefore, that the estimate for growth is a minimum whereas the estimate for mortality is a maximum. The true growth rate for the population during the study period was probably intermediate between these estimates, perhaps 820 and 14,000 mg/m^2/yr for the body and shell, respectively. The 620 mg/m^2 lost by the population during the spawning season will be considered an approximation of the quantity of gametes produced, although this is probably a minimal estimate.

Energy Flow

The respiratory rate of *Modiolus* was a function of both size and temperature (Fig. 6). The regression coefficient of observations plotted logarithmically is b, the exponent of the equation $O_2 = aW^b$, where O_2 is the oxygen consumption, W is the weight, and a is a constant. Analysis of the biological meaning of various values of b has been well reviewed by Zeuthen (1953) and Bertalanffy (1957). The fact that respiratory rates for medium sized mussels (0.3–0.6 g) were lower in air than in water may be partly the result of a lower metabolic rate, since they were not filtering and feeding. The convergence of the regression lines in air at about 0.5 g body weight suggest that respiration may also be limited by surface exchange in mussels larger than this size. Since a mussel may be in a small pool of water at low tide, its respiratory rate may be limited, at least part of the time, by the rate of solution of oxygen from air rather than the mussel's ability to extract oxygen from either medium. There is probably no permanent damage due to insufficient oxygen uptake during low tide because mussels, in common with many other lamellibranchs, can endure anaerobiosis for extended periods. Two mussels lived for 10 days at 10°C in the laboratory

TABLE 8. *Flux rates of energy in a* Modiolus *population, in kg-cal/m²/yr*

Growth: Body	3.7	
Shell	8.4	
Byssus	1.8	
Reproduction (gametes):	2.8	
TOTAL PRODUCTION		16.7
Respiration: Water	11	
Air	28	
TOTAL RESPIRATION		39
ASSIMILATION		56

with their valves clamped tightly closed; the maximum survival time without oxygen was not determined. Mussels which were permitted to gape have lived out of water for 28 consecutive days during June at Sapelo Island when protected from direct sunlight.

The Q_{10} of respiration in water was very nearly 2, but from the regressions in air the Q_{10} appeared to be greater than 2 for mussels less than 0.12 g and decreased to unity at about 0.5 g. Since the decrease in apparent Q_{10} may only be the result of an oxygen debt which will be satisfied in nature during the next flood tide, it is assumed in this paper that the $Q_{10} = 2$ for both air and water.

The rates of energy flow through the average mussel population in the marsh may now be calculated (Table 8). The organic matter of the shell and byssus were assumed to be pure protein with a caloric content of 5.7 kg-cal/g. Three analyses of body tissues yielded 4.48 ($s = 0.09$) kg-cal/g, and gametes were assumed to have this same energy content. In spite of the huge amount of energy, relative to the active tissues of the body, which goes into formation of shell and byssus, the growth efficiency (growth/assimilation) is 25%. Slobodkin (1959) states that growth efficiencies of 20–28% may be considered almost universal. Reproductive efficiency for *Modiolus* is 5.0%; this also agrees with Slobodkin's (1959) suggestion that reproductive efficiency is often slightly less than the square of the growth efficiency. The values for annual respiration should be considered only first approximations because of three shortcomings of the data. The fact that respiration at low tide may depend on the solution of oxygen into tide pools has already been mentioned. Secondly, seasonal changes in respiration were not measured; respiration at other seasons was extrapolated from the rate and Q_{10} at one time of year and the mean monthly temperatures. Finally, respiratory rates were measured over only a limited range of sizes and the curves were extrapolated to include larger and smaller animals. It is noteworthy that this marine invertebrate apparently performs more than half its total respiration while exposed to the air.

DISCUSSION

The cause of the low population densities in Short-*Spartina*-High-Elevation marsh and *Salicornis-Distichlis* marsh (types 5 and 6) compared to Medium-*Spartina*-Levee marsh and *Juncus* marsh (types 3 and 7) is not known, but the dense vegetative cover of the latter two types may serve as a shield against the hot summer sun or produce a firm base for attachment of the byssi and thereby help reduce predation losses. Mechanisms promoting survival may more than compensate for slower growth due to less abundant food at higher elevations. The diseases and predators which cause natural mortality in the ribbed mussel are largely unknown. Wells (1958a, b) found the gastropods *Fasciolaria hunteria* and *Murex fulvescens* capable of opening and eating *Modiolus* but these predators do not occur in the marsh near Sapelo Island. Blake (1960) found a direct correlation between oxygen consumption of *Modiolus* (which was a function of age, growth rate, and feeding) and its attractiveness to *Urosalpinx cinerea*. However, *U. cinerea* was not found in the marsh at Sapelo Island, nor were drilled shells of *Modiolus* found there. The raccoon, *Procyon lotor*, is abundant in the marsh and is probably an important predator.

The reported setting and growth of *Modiolus* on piles at Beaufort, North Carolina (McDougall 1943), conflicts with pres-

ent observations. At Sapelo Island, *Modiolus* is found in the salt marsh, and *Brachidontes exustus* lives on piles at intertidal levels. Since *B. exustus* was not mentioned by McDougall, even though it occurs in North Carolina, he may possibly have mistaken the pile-dwelling mussels for *Modiolus*.

Coe and Fox (1942) reported mean shell lengths for one, two, three and four-year old *Mytilus californianus* to be 70, 105, 123 and 131 mm, respectively. Coe (1945) reported maximum measurements in *Mytilus edulis diegensis* to be 76 and 96 mm at the end of the first and second years of growth. Since the length-to-height ratio of *Modiolus* is 2.5:1, and since Figure 5 indicates it did not reach 20 mm height in less than two years, *Modiolus* had a lower growth rate than these other mussel species. The growth rate of *Modiolus*, however, does compare well with the average rates for *Mytilus edulis* as reported or reviewed by Field (1922) and Richards (1935), and is higher than the average rates reported for *M. edulis* by Mossop (1923). It must be remembered that these examples of growth rates in various mussel species are not strictly comparable since the populations differ both genetically and in habitat conditions. Furthermore, Mossop (1923) found that the growth rate of *M. edulis* varied markedly from year to year and that favorable or unfavorable conditions determined the rate of growth irrespective of preceding conditions. Therefore the rate of growth of *Modiolus* presented here, based on only one year of study, should not be assumed to be an average rate applicable to other years or to other localities.

The large amount of organic turnover due to growth, mortality and respiration of the population is a strong argument for considering mussels a dominant population from the functional as well as the biomass standpoint. Odum and Smalley (1959) discussed the energy flow of two other important populations in this same marsh, grasshoppers (*Orchelimum fidicinium*) and snails (*Littorina irrorata*). The grasshoppers were most abundant in the taller grass of the low marsh and consume the living *Spartina*, whereas the snails were abundant in the Short-*Spartina*-High marsh and feed on marine algae and detritus deposits. They determined the annual flux rates to be 28 and 290 kg-cal/m^2 for the grasshoppers and snails, respectively, in those marsh types most favorable for each population. However, for the entire marsh the energy flow per unit area of these two populations is considerably smaller. The flux of the snails probably is not much different from that of the mussels, whereas that of the grasshoppers is much less than either.

Analysis of the total biological energy flow of the salt marsh is currently being prepared by another writer. His preliminary report (Teal 1959) for the crab, nematode, insect, bird, and mammal populations showed that each has the same order of magnitude energy flow as the mussels, whereas the bacteria may have one or two orders of magnitude greater flow. Therefore the mussels are important not only because they occupy the filter-feeding niche in the marsh, but also because they are a true functional dominant.

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ON THE RESPONSE TO CHANGING TEMPERATURE OF THE TEMPERATURE-LIMITED PLANKTER *DOLIOLUM DENTICULATUM* QUOY AND GAIMARD 1835[1]

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ABSTRACT

D. denticulatum is limited at the lower end of its temperature range by temperature values of about 14° to 15°C. In the Pacific it inhabits the subtropical anticyclonic gyres and the area between them. It is carried southeastward past California and Baja California and is spread into the California Current system by horizontal mixing. It survives where the water carries it if temperatures are suitable. The seasonal movements of the isotherms are so rapid that the organism does not at any time inhabit all favorable areas and it may be overtaken in some months by unfavorable environments. In the period from 1949 through 1955, its seasonal movement was to the north in summer and to the south in winter. Its range of spatial fluctuation was less than that of its critical temperature. In the warmer period beginning in 1957, it has lived closer to the coast in late fall and has been more generally distributed in the cooler part of its range than in the earlier years. It has been carried northward by the winter coastal countercurrents and southward again in late spring with the cold upwelled water. The direction of its seasonal fluctuation has thus been reversed in the warmer years.

INTRODUCTION

During the latter part of 1957, the temperature in the upper mixed layer of the California Current system rose approximately 1°C above the average of the previous 8 years and remained high through December of 1959 at least. This presented an opportunity to test the temperature reponses of various organisms to a new environment. One of these organisms, *Doliolum denticulatum*, has proved especially interesting. It is the purpose of this paper to describe the general distribution of the species, its temperature limitations and its normal seasonal fluctuations in the earlier years and to discuss its peculiar responses during the latter period.

METHODS

The plankton samples and the hydrographic data used in this study were collected off the west coast of California and Baja California, Mexico, on cruises of the California Cooperative Oceanic Fisheries Investigations (CCOFI) and on other cruises in the region. The nets used in the collection of the plankton samples were made of No. 30 xxx grit gauze (0.65 mm mesh size), conical in shape, with a one-meter mouth opening. The last 40 cms and cod-end bags were made of No. 56 xxx grit gauze (0.31 mm mesh size). Tows were oblique from either 70-m depth (1949–50) or 140 m (after 1950). Each net was equipped with a flow meter, which allowed the amount of water filtered to be calculated. Detailed descriptions of the nets, methods used in making the plankton tows and generalized station patterns are given by Ahlstrom (1948, 1952 and 1954).

Extensive plankton sampling in the area under consideration was started in March of 1949. There were very few data or samples available previous to that time. The NORPAC Cruise (NORPAC Committee 1960) made in August and September 1955 has been used to establish the offshore distributions beyond the usual area sampled by CCOFI cruises.

All of the samples from 24 of the CCOFI cruises, alternate samples from 6 others, and samples from the pertinent areas from 10 others have been examined. In all, samples from 2,961 stations, not including the NORPAC material, have been examined. *D. denticulatum* was present at 1,230 of them.

[1] Contribution from the Scripps Institution of Oceanography: New Series.

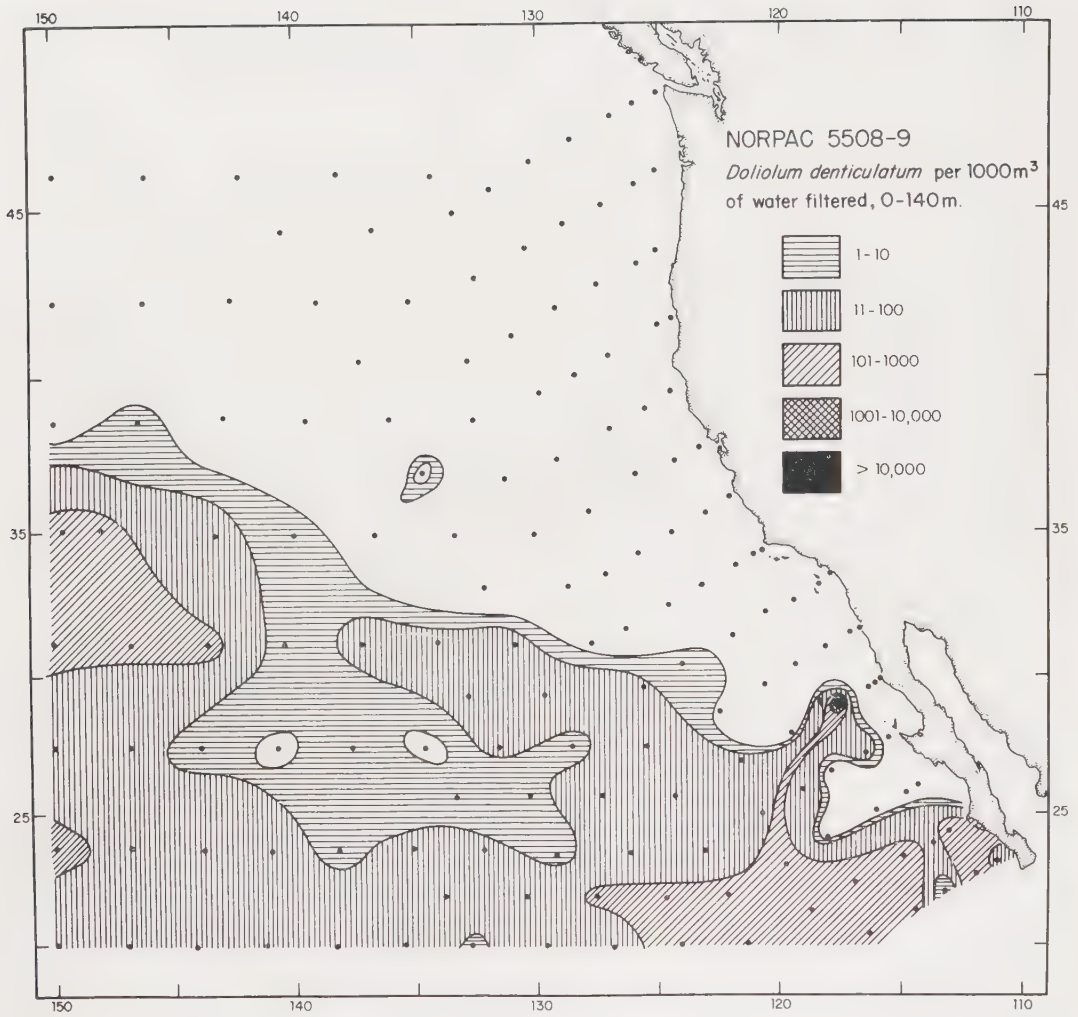

FIG. 1. Distribution of *Doliolum denticulatum* in August and September 1955 (the NORPAC expedition).

HORIZONTAL DISTRIBUTION

D. denticulatum has been found in subtropical waters near Australia (Thompson 1948), in the western equatorial Pacific by the Siboga expedition (Ihle 1910), in the Kuroshio (Tokioka 1937), north of Hawaii in the NORPAC collections of the Bureau of Commercial Fisheries Honolulu Biological Laboratory (unpublished data), in SHELLBACK sections across the equator (unpublished data) and in the area between the Californias and Hawaii (Fig. 1). These observations indicate that the species inhabits the subtropical anticyclonic gyres of the North Pacific (Kuroshio, Westwind Drift, California Current, and North Equatorial Current) and the South Pacific (East Australia Current, Westwind Drift, Peru Current, and South Equatorial Current) and the waters between these two gyres. *D. denticulatum* moves with these systems, constantly spreading outward from its permanent central concentrations. This study is limited to the California Current system; the NORPAC data (Fig. 1) are sufficient to describe the pertinent part of the general habitat of *D. denticulatum*. The long series of CCOFI data can be used to

TABLE 1. *Vertical distribution of* D. denticulatum *at 79 stations; numbers per 1000 m³ of water filtered*

Depth (m)	Min. number	Quartile based on 0–131 m			Max. number
		1st	2nd	3rd	
0–131	2	14	38	133	34,617
131–262	0	0	0	0	20

Whenever *D. denticulatum* was present in a deep tow it was also taken in the associated upper tow.

TABLE 2. *Vertical distribution of* D. denticulatum *(average of 6 stations)*

Depth (m)	No. of samples	No/1000 m³	Temp. (°C)
0–25	6	97	18.8
25–50	5	34	19.1
50–75	5	18	18.2
75–100	6	12	17.7
100–300	3	2	11.0

examine its temperature dependence in that area.

VERTICAL DISTRIBUTION

All data where depth range can be determined indicate that *D. denticulatum* is found primarily in the upper 100 m of the water column, with the major portion of the population in the upper 50 m. The vertical distribution of the species found during the NORPAC Cruise is given in Table 1.

The vertical distribution of the species is shown in more detail by an analysis of 6 stations taken on a cruise southwest of San Diego in May 1954. Three of these stations were occupied during daylight hours and three at night.

The species is small, ranging up to 8 mm, and its swimming movements at best can be described as weak. It is also restricted to the shallow depths (Tables 1 and 2), mostly above the depth of the winter thermocline, and as an indicator of surface flow may be better than those organisms which undergo extensive vertical migration or have a greater depth range.

THE LIMITING EFFECT OF TEMPERATURE

The subtropical and shallow distribution of *D. denticulatum* suggests that temperature may be limiting. Since the organism is most abundant in the upper mixed layer we have chosen the temperature at 10 m below the surface as an index of its environment. The value at 10 m differs from the value at the surface by small amounts near the coast in the late summer months. Later we shall refer to anomalies of surface temperature, since data in this form are more readily available and since these anomalies have been shown to extend throughout the mixed layer (Reid 1960).

The CCOFI cruises cover an area with a broad range of temperature, but the nature of the program is such that, at most of the stations taken, the 10-m temperature lies between 12 and 19°C (Fig. 2). In the cruises discussed here, the greatest numbers of stations were found in the 14–15°, 15–16° and 16–17° ranges, but the greatest percentages of stations at which *D. denticulatum* was found were at higher temperatures. If one considers the ratio of stations at which it was found to all stations in each 1°C-interval (Fig. 3) there is a rapid rise between 12 and 16°C and above 16°C the ratio is consistently high. *D. denticulatum* is found in much warmer water, and these

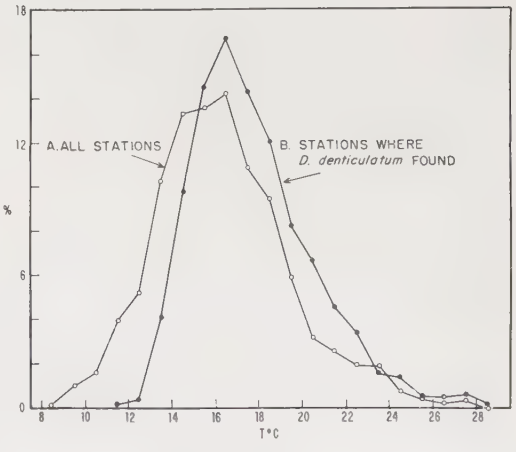

FIG. 2

A. Classification of the samples by the temperature at 10 m depth at the sampling position. The ordinate is the percentage of the 2,961 samples within the 1°C classes.

B. Classification of the 1,230 samples at which *D. denticulatum* was found.

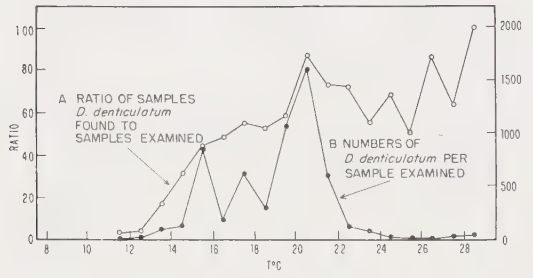

FIG. 3

A. Ratio of samples in which *D. denticulatum* was found to samples examined.

B. Average numbers of *D. denticulatum* per sample per 1,000 m^3.

data do not suggest an optimum or an upper limiting temperature.

The numbers of *D. denticulatum* per station in the 1°C-intervals, and the ratio of stations where the organism is found appear to be unrelated to temperature above 22°C. In that range the concentrations are low but the ratios are high. This may be taken to imply that the species, though generally distributed in the region of high temperatures (most of the high temperature data are from south of Punta Santa Eugenia), is limited in abundance there by other factors.

We believe, however, that the species is limited by temperatures less than about 14–15°C. The lowest 10-m temperature at which *D. denticulatum* was found was 11.8°C, but rather small numbers are found below 14–15°C (Fig. 3).

MOVEMENT OF THE ISOTHERMS

A planktonic organism moves with the water and survives where conditions are suitable. If one environmental factor alone limits an organism then the distribution of that factor will describe the areas favorable for its survival. Organisms, however, will not enter suitable areas if currents are adverse or if the favorable conditions appear and disappear too rapidly. The seasonal movements of isotherms in the mixed layer, for example, are in most cases very rapid compared to processes of advection and diffusion and may have a limiting effect on organisms.

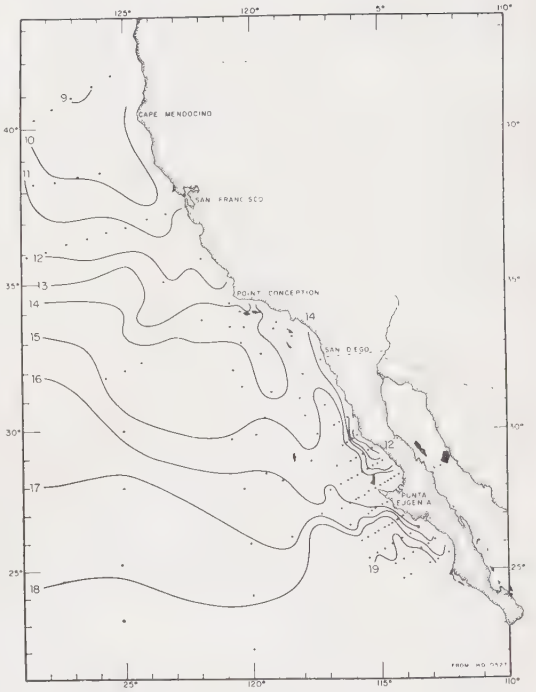

FIG. 4. Temperature at a depth of 10 m in March based on data from several years.

The California Current system offers certain curious illustrations of the combined effects of the seasonal changes of current and temperature. The currents well offshore generally flow southeastward and carry water colder and less saline than that to the west. Between 25° and 35°N a tongue of colder water is generally found extending southeastward (Figs. 4 and 5). The inshore currents flow southeastward from March through October and northwestward from November through February. Isotherms in these latitudes normally move northward during the period of high radiation from March through September and southward in the other months. Upwelling, which occurs north of 35°N in summer, reduces the coastal temperatures well below the latitudinal average. The coastal currents flow north during the period of falling temperatures and south during the period of rising temperatures. The 10-m temperatures in March and August are illustrated in Figures 4 and 5. A rapid northward movement of some iso-

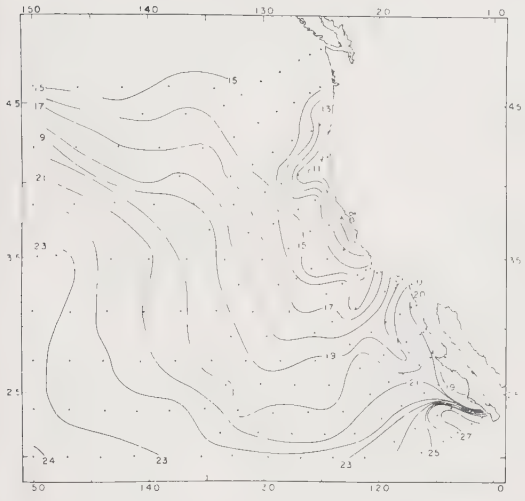

FIG. 5. Temperature at a depth of 10 m in August, 1955. Data are from the CCOFI part of the NORPAC expedition.

therms is indicated. That part of the change from May to August is faster still. The coastal end of the 18°C isotherm, for example, moves northward at about 6 miles per day. If the southerly flow of the water is taken conservatively as a quarter-knot, the average speed of the isotherm through the water is 12 miles per day during three months.

Conversely, the most rapid southerly movement of the 18°C isotherm occurs as the result of the winter cooling of the surface waters from November to February. This movement amounts to about 4 miles per day although the water is moving northward near shore at that time of year.

SEASONAL VARIATION OF *D. DENTICULATUM*

D. denticulatum normally lives in the southwestern part of the California Current system. It is continually supplied to the area from the northwest and swept away to the south by prevailing currents. As in any concentration the processes of horizontal mixing tend to spread the species outward from the permanent concentrations to the west. As inshore areas become favorable in temperature, for example, the species will tend to spread into them. If the areas change in temperature rapidly the rate of mixing and flow may not be able to keep up, so that at any time there may be uninhabited favorable areas or areas which have become unfavorable, but from which the species has not yet been entirely eliminated.

In such a regime the rapid seasonal movement of the isotherms may cause large monthly shifts in the boundaries of the favorable environment, and the seasonal adjustments of the species may lag.

In the period from 1949 through 1955, *D. denticulatum* showed fairly regular seasonal changes. In 1949, it was least abundant and farthest south in March and most abundant and farthest north in September (Fig. 6A, B, C,). If one compares the distributions with the position of the 14°C isotherm at a depth of 10 m in the various months it may be seen that in March (approximately the seasonal minimum of temperature) the northern limit of the species is aligned rather closely with this temperature but has been slightly over-run by the rapid southerly movement of the isotherm. (The position of the 14°C isotherm is determined not only from the hydrographic station data where the net tow was made but from the intermediate bathythermograph data as well. The isotherm therefore may have a more complex shape than the other isopleths.)

Between March and June upwelling begins with a strong offshore movement of the surface waters north of Point Conception. This opposes any rapid northward movement of *D. denticulatum*, although the increased radiation has raised the temperature and moved the 14°C isotherm well to the north. By September the processes of horizontal mixing have extended the northern distribution more than 200 miles closer to the coast where it takes advantage of part of the vast area of warmer water which has appeared since March. It is presumed that the upwelled water that continues to flow southward from the area above Point Conception represents both a velocity and low-temperature barrier, which the species usually cannot cross to reach the warmer inshore waters.

The most northward coastal observation of *D. denticulatum* in the period 1949–1955

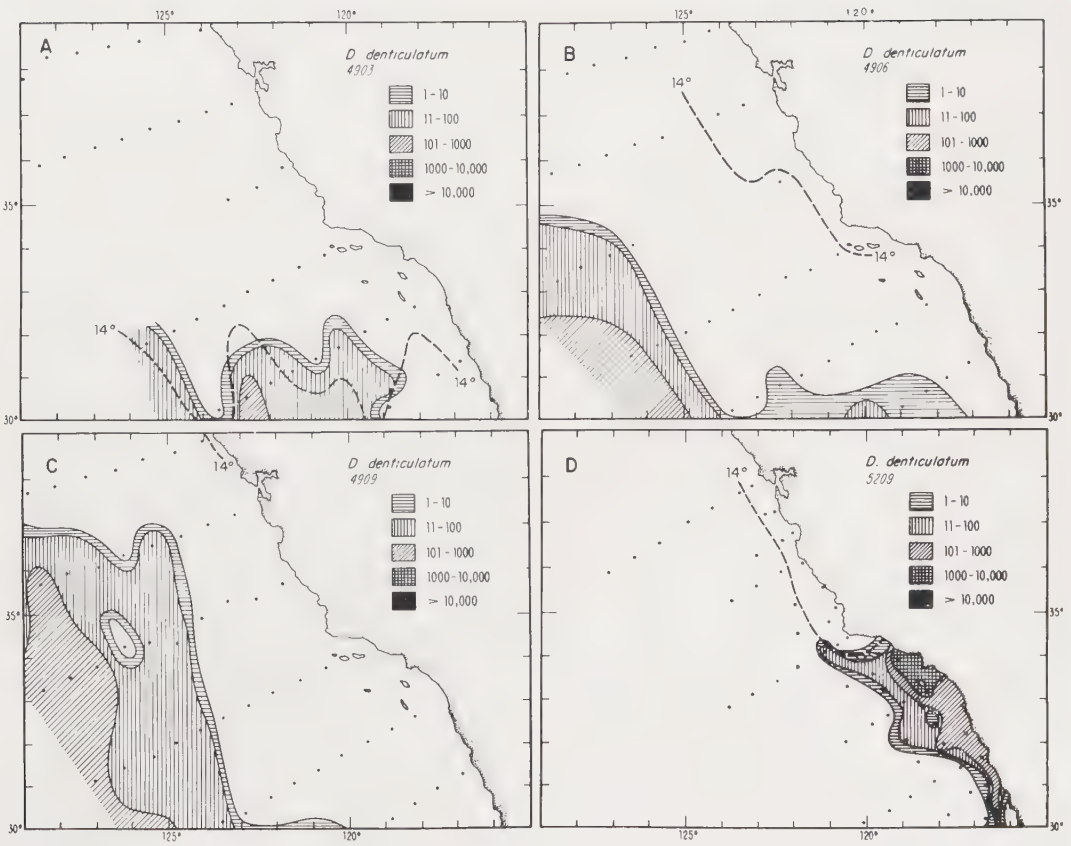

FIG. 6. Distribution of *D. denticulatum* in March (4903), June (4906) and September (4909) of 1949 and in September of 1952 (5209). Units are numbers per 1000 m^3 of water filtered. The dashed line represents the position of the 14°C isotherm at a depth of 10 m.

was in September of 1952, when it reached Point Conception (Fig. 6D). The temperature, however, was already falling at this time and no specimens were found in the October samples from that area.

The small extent of the seasonal fluctuations described by Figure 6 may be only the consequence of this particular system, in which seasonal fluctuations of coastal currents and temperature are in opposite phase. The species cannot adjust as rapidly as the changing environment and is constantly being overtaken by favorable or unfavorable conditions. Its northward movement in summer and its southward movement in winter are further retarded by the motion of the water, which is most strongly southward and offshore in summer and least so in winter.

NON-SEASONAL VARIATIONS OF TEMPERATURE

The years from 1949 through 1956 were of course not identical in temperature, in distribution of organisms or in perhaps any other item measured by CCOFI. Comparison with previous periods (mostly shore station data) and subsequent CCOFI measurements suggest that with regard to temperature these 8 years had certain common features which distinguished them from other years and from the long-term mean which was determined from the years 1920–1938 (Reid, Roden and Wyllie 1958). From 1949 through 1956 the spring temperatures were lower than the mean by about 0.5°C and the late fall values were higher than the mean by a smaller amount all along the coast between 25° and 40°N.

FIG. 7. Surface temperature anomaly in January 1958. (Surface temperature in January 1958 less the mean January temperature 1949–1954.)

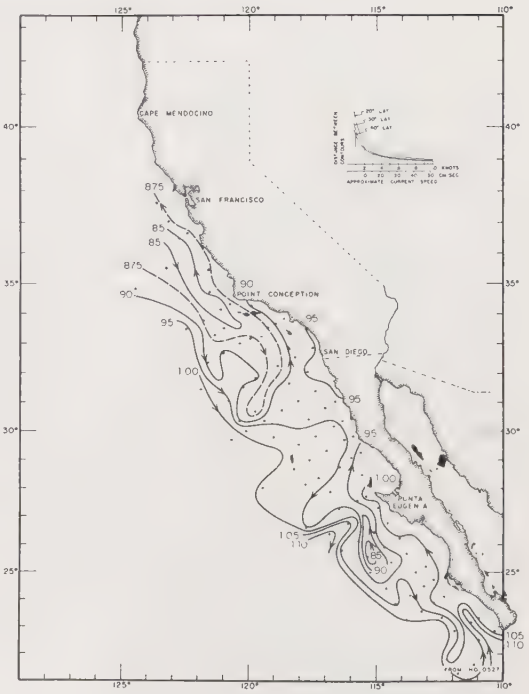

FIG. 8. Geostrophic current in January 1958. (Geopotential anomaly of the zero-decibar surface with respect to the 500-decibar surface, in dynamic meters. The velocity scale is drawn for a contour interval of 0.05 dynamic meters.)

Since the mean values were from coastal data a new mean was defined from the CCOFI cruises for the years 1949 through 1954, and the years since 1956 have been compared with it, offshore as well as at the coast. Compared with this 1949–1954 mean, the late 1957 and earlier 1958 surface temperatures were found to be high by about 1°C (Reid 1960) and later unpublished data reveal that this period of high temperature has persisted at least through December 1959.

This anomaly is concurrent with certain changes in the circulation that might also be important to *D. denticulatum*. Upwelling north of Point Conception was reduced in the summers of 1958 and 1959 and the countercurrent was more strongly developed in winter (Reid 1960). The temperature anomaly from the 1949–1954 January mean and the geostrophic flow and drift bottle recoveries for January 1959 are shown in Figures 7, 8 and 9.

NON-SEASONAL VARIATIONS OF *D. DENTICULATUM*

The effect of these changes in temperature and circulation in redefining the favorable environment has been not only to bring *D. denticulatum* farther inshore but to allow it to extend across the colder tongue and in to the coast at about 32°N. It not only survived inshore of the Channel Islands (Fig. 10) but advanced northward with the winter countercurrent as far as San Francisco Bay in January. It remained far north until the countercurrent ceased and then was swept slowly south and west as upwelling began (Figs. 10 and 11).

Among the March samples, the 4 innermost of the northern line included larvae of the intertidal sand crab *Emerita analoga*, which must have come from very close inshore. They indicate an offshore movement of water (Johnson 1939). The *D.*

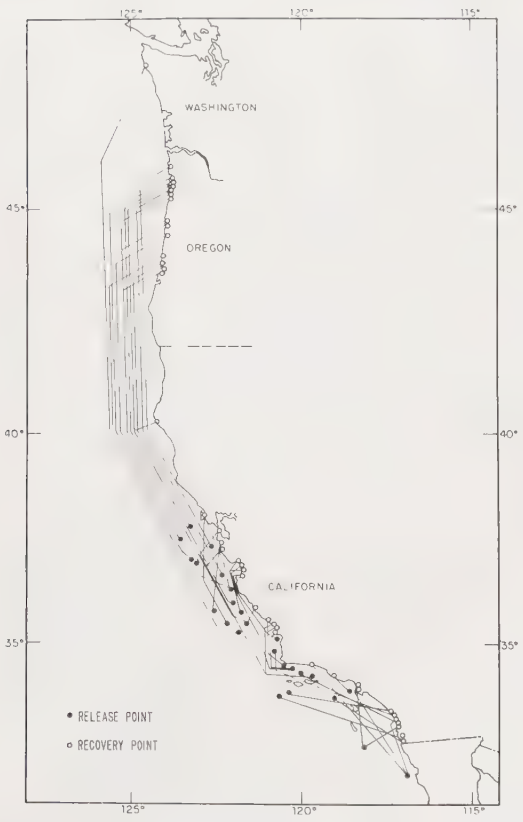

FIG. 9. Recovery points of drift bottles released in January 1958.

denticulatum specimens may have come either with these larvae from farther north (where they were found in January) or eastward by mixing from the offshore concentration.

Ordinarily it would seem curious that a temperature-limited plankter should move northward in winter, as temperatures fall, and southward as temperatures rise. In the earlier years (1949–1955) no such movements were observed. *D. denticulatum* was usually kept offshore by the current and the cold tongue; when it did cross the tongue, as in September of 1952, it could not survive more than a month.

In the presence of the warmer water of 1957–58, *D. denticulatum* crossed the area usually occupied by the cold tongue, survived and was later carried northward by the countercurrent. As upwelling began the northern part of the population did not retreat to the southwest but was swept away from the coast and to the south (Figs. 10 and 11). This same phenomenon may explain part of the offshore distribution found in March. The species was thus carried from the northern area in May and June even though the temperatures remained high.

The period of high temperature in the California Current persisted at least through December of 1959. This allowed the hypothetical relation between *D. denticulatum* and the temperature and currents to be tested twice more. Not all of the samples from every cruise since June of 1958 have been examined, but a check of samples taken in October 1958, January, October, and November of 1959 has found *D. denticulatum* in the inshore area north of Point Conception in each month.

Although temperatures north of Point Conception have risen in the latter period, they are still relatively low in terms of the animal's range. The northward movement of the animal has in fact changed its distribution with temperature. In Figure 12 the ratio of samples with *D. denticulatum* to total samples is plotted separately for the period 1949–1955 (2,207 samples, 747 with the animal present) and for 1957–1958 (754 stations, 483 with the animal present). A marked rise in the ratio is seen near the cooler part of the range in the latter period, but the range is not extended nor its previous limit reached. Curiously, the animal has been more generally distributed in the colder water in the warmer years. The authors suggest that this also is a consequence of the warming and weakening of the cold tongue south of Point Conception, which has allowed the animal to extend into the seasonally northward-flowing water, whose temperature, though low, is not beyond the range tolerable to the animal.

SUMMARY

D. denticulatum inhabits the central subtropical anitcyclonic gyre, which includes the outer part of the California Current. The species is maintained in the areas offshore from California and Baja California by this system, and is carried inshore by

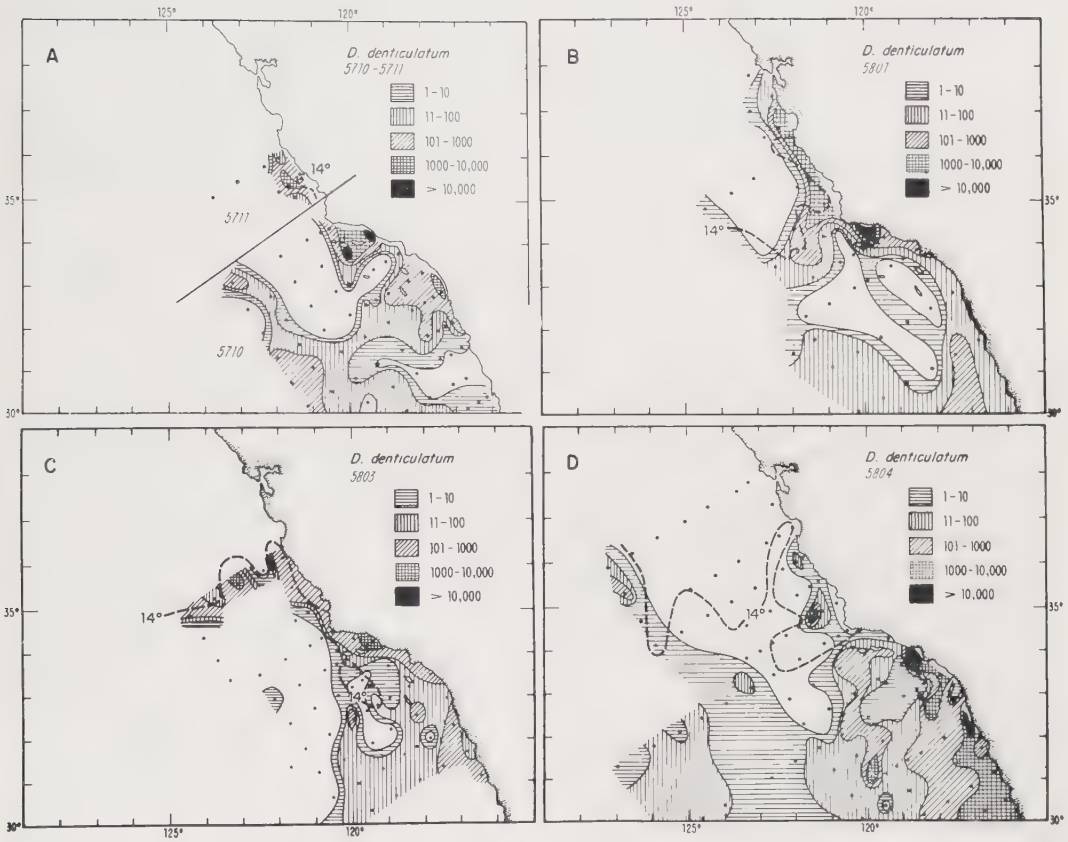

FIG. 10. Distribution of *D. denticulatum* in October–November 1957 (5710–5711), January 1958 (5801), March 1958 (5803) and April 1958 (5804). Units are numbers per 1000 m^3 of water filtered. The dashed line represents the position of the 14°C isotherm at a depth of ten meters.

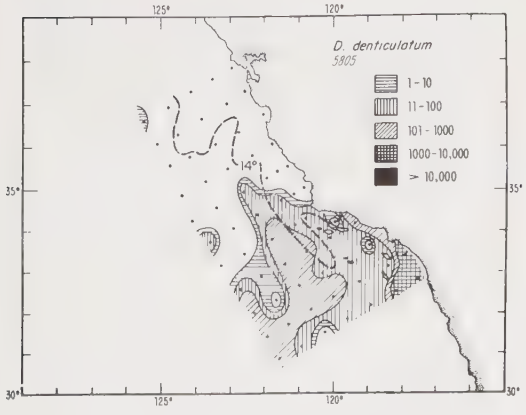

FIG. 11. Distribution of *D. denticulatum* in May 1958 (5805). Units are numbers per 1000 m^3 of water filtered. The dashed line represents the position of the 14°C isotherm at a depth of ten meters.

horizontal mixing processes. It survives in such favorable areas as it enters, but the areas of favorable environment fluctuate so rapidly with season that at any time the organism may not inhabit all of the favorable areas. It may also be overtaken by unfavorable conditions. It is severely limited by low temperature (about 14° to 15°C) and it is the rapid movement of isotherms near these temperatures which accounts for the environmental changes. The species' limited ability to adjust its position with these changes probably accounts for its rather small seasonal fluctuation, since in the greater part of the year the coastal waters move in a direction opposite to the motion of the isotherms.

The species can, however, be distributed into regions that remain favorable for sev-

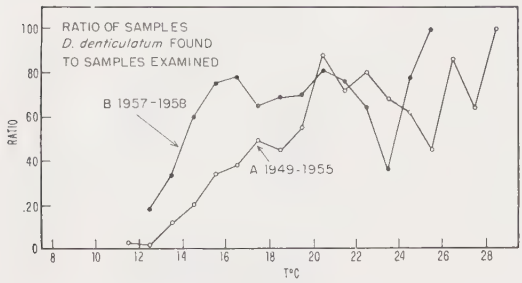

FIG. 12

A. Ratio of samples in which *D. denticulatum* was found to samples examined in the period 1949–1955.

B. The ratio in 1957–1958.

eral months, and the winter seasons of 1957–58, 1958–59 and 1959–60 represent longer period changes of environment to which the organism has more successfully adjusted. Its northward movement has not been into water colder than its previous range, but the northern water does represent the cooler part of its range. Its distribution in the warmer period has thus been shifted toward the cooler part of its range.

D. denticulatum is of particular interest as an example because of its critical temperature limit. Temperatures in the California Current system have changed in the period 1957–59 in such a fashion that inshore the seasonal fluctuation of the species has reversed, and it is farthest north in winter. Organisms critical to higher temperatures might not show this reversal; organisms critical to lower temperatures might show it every year.

ACKNOWLEDGMENTS

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The authors are indebted to Mr. G. I. Murphy and to Dr. E. H. Ahlstrom for various suggestions and for the interest they have shown in discussing various points.

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NOTES AND COMMENT

Accumulation of Toxaphene by Fish-food Organisms[1]

Toxaphene (octachlorocamphene) has been evaluated by many investigators for its piscicidal properties. Their results indicate that toxaphene has definite possibilities as a fish toxicant. Initial toxicity is high, but in many instances the residual period is extremely long. In several cases the findings suggest that toxaphene may have been accumulated by fish-food organisms at a time when treated waters were still toxic to fish. Some basis for this assumption lies in the fact that during the later stages of the detoxification of the water, caged fish showed no indication of

[1] A portion of a thesis submitted to the Graduate Faculty of Colorado State University, by the senior author in partial fulfillment of the requirements for the degree of Master of Science.

Table 1. *Bioassays* of planktonic animals* (Daphnia magna *and* D. pulex) *exposed to a single, sublethal toxaphene dosage of 0.03 ppm*

Trial no.	Toxaphene percentage and formulation	Exposure period (hr)	Species of *Daphnia*	Approx. no. of *Daphnia*	No. of fish	TEST FISH							CONTROL FISH			
						No. of fish surviving after (hr)						Av. water temp. (°F)	No. of fish surviving after (hr)			Av. water temp. (°F)
						24	48	72	96	120	144		96	120	144	
1	100% technical grade	36	*pulex*	3,780	4	4	4	4	4	4	–	73	4	4	–	72
2	40% wettable powder	72	*pulex*	6,000	4	4	4	4	4	4	–	70	4	4	–	69
3	100% technical grade	96	*pulex*	4,500	4	4	4	4	4	–	–	71	4	–	–	70
4	40% wettable powder	192	*magna*	4,980	4	4	4	4	4	4	4	73	4	4	4	72
5	100% technical grade	216	*pulex*	4,900	4	3	3	3	2	2	1	73	4	4	3	73
6	100% technical grade	216	*pulex*	4,800	4	4	4	4	4	4	4	70	4	4	4	71
7	100% technical grade	288	*pulex*	4,500	4	4	4	4	4	4	4	70	4	4	4	71
8	100% technical grade	312	*magna*	4,500	4	4	4	4	4	4	4	71	4	4	4	71
9	100% technical grade	312	*pulex*	4,800	4	4	4	4	4	–	–	72	4	–	–	70
10	40% wettable powder	408	*magna*	3,990	4	4	4	4	4	4	4	73	4	4	4	72
11	100% technical grade	624	*magna*	12,000	4	4	4	4	4	4	4	70	4	4	4	70

* *Oncorhynchus nerka kennerlyi*; employed as test fish.

TABLE 2. *Bioassays of a planktonic animal* (Daphnia magna) *exposed to multiple, sublethal dosages of toxaphene*

Trial no.	Daily and total dosage of toxaphene (ppm)	Toxaphene percentage and formulation	Exposure period (hr)	Species of fish	No. of fish	TEST FISH: No. of fish surviving after (hr) 24	48	72	96	120	TEST FISH: Av. water temp. (°F)	CONTROL FISH: No. of fish surviving after (hr) 96	120	CONTROL FISH: Av. water temp. (°F)
1	0.01 0.13	100% technical grade	312	*O. nerka kennerlyi*	4	0	0	0	0	0	68	4	4	69
2	0.01 0.13	40% wettable powder	312	*O. nerka kennerlyi*	4	0	0	0	0	0	69	4	4	69
3	0.01 0.1	100% technical grade	240	*Notropis* sp.	6	6	2	2	2	2	69	6	6	70
4	0.01 0.1	40% wettable powder	240	*Notropis* sp.	6	3	2	1	0	0	69	6	6	70
5	0.02 0.1	100% technical grade	120	*Notropis* sp.	6	5	4	3	3	3	72	6	6	73
6	0.02 0.1	40% wettable powder	120	*Notropis* sp.	6	5	3	2	1	0	72	6	5	73

toxicity, whereas reintroduced fish populations died.

A study was conducted in the Zoology Department at Colorado State University from September, 1957, to June, 1959, to determine whether accumulations of toxaphene could occur in fish-food organisms.

In controlled laboratory experiments sublethal concentrations of toxaphene were employed to simulate the detoxification of treated water below the level of lethality to fish-food organisms. The sublethal concentration of 100% technical grade toxaphene dissolved in isopropyl alcohol was determined for three species of fish; green sunfish, *Lepomis cyanellus*, kokanee salmon, *Oncorhynchus nerka kennerlyi*, and shiners, *Notropis* sp. A sublethal concentration of the same toxaphene formulation was also determined for the cladocerans, *Daphnia pulex* and *D. magna*, and the damselfly nymphs, *Ischnura* sp. and *Enallagma* sp.

A sublethal concentration was defined as that concentration which produced no greater mortality among the test animals than was sustained by the controls. These

TABLE 3. *Bioassays* of periphyton* (Cladophora *spp.*, Anacystis *spp.*, Scenedesmus *spp.*, Navicula *spp.*, Diatoma *spp., and* Ciliophora) *exposed to multiple toxaphene dosages*

Trial no.	Daily and total dosage of toxaphene (ppm)	Toxaphene percentage and formulation	Exposure period (hr)	No. of fish	TEST FISH: No. of fish surviving after (hr) 24	48	72	96	120	TEST FISH: Av. water temp. (°F)	CONTROL FISH: No. of fish surviving after (hr) 96	120	CONTROL FISH: Av. water temp. (°F)
1	0.01 0.13	100% technical grade	336	10	0	0	0	0	0	70	10	10	70
2	0.01 0.13	40% wettable powder	336	10	0	0	0	0	0	70	10	10	70

* *Notropis* sp. employed as test fish.

TABLE 4. *Bioassays* of a planktonic alga* (Scenedesmus incrassatulus) *exposed to multiple toxaphene dosages*

Trial no.	Daily and total dosage of toxaphene (ppm)	Toxaphene percentage and formulation	Exposure period (hr)	No. cells/L	No. of fish	TEST FISH: No. of fish surviving after (hr) 24	48	72	96	120	TEST FISH: Av. water temp. (°F)	CONTROL FISH: No. of fish surviving after (hr.) 96	120	CONTROL FISH: Av. water temp. (°F)
1	0.01 0.16	100% technical grade	384	$1{,}850 \times 10^5$	6	6	6	6	6	6	70	6	6	70
2	0.01 0.16	40% wettable powder	384	$3{,}300 \times 10^5$	6	6	6	6	5	5	70	6	6	70

* *Notropis* sp. employed as test fish.

sublethal concentrations were as follows: green sunfish, 0.0036 ppm (after 96 hr); kokanee salmon, 0.0036 ppm (after 96 hr); shiners, 0.01 ppm (after 96 hr); *D. pulex*, 0.03 ppm (after 168 hr); *D. magna*, 0.03 ppm (after 120 hr); damselfly nymphs, 0.004 ppm (after 96 hr). The dissolved oxygen and free carbon dioxide content of those solutions containing fish were determined, and at no time dropped below 4.0 ppm of oxygen nor exceeded 12.0 ppm of carbon dioxide. Water temperatures varied between 68° and 73°F.

Accumulation studies in the laboratory involved the bioassays of four aquatic communities which were exposed to single, 0.03 ppm, and chronic, 0.01 and 0.02 ppm applications of toxaphene. The communities were: 1) planktonic animals, 2) planktonic algae, 3) periphyton and 4) insect nymphs. Forty per cent wettable powder toxaphene, as well as the previously described 100% technical grade formulation, were employed. The above named fish were utilized as test fish. In each bioassay a comparable, unpoisoned quantity, the community under test, was added to the control aquarium.

The results of the laboratory bioassays indicated that *Daphnia* probably did not accumulate toxaphene to a toxic level when exposed to a single, sublethal dosage of 0.03 ppm for periods of 36 to 624 hr (Table 1). However, a mortality among the test fish did occur when *Daphnia* were exposed to daily, sublethal dosages of 0.01 and 0.02 ppm of toxaphene over periods of 120 to 312 hr (Table 2). The latter bioassays also suggested that *Daphnia*, which were exposed to toxaphene for the greatest length of time, produced a quicker and higher mortality when fed to the test fish.

Cultures of the planktonic alga, *Scenedesmus incrassatulus*, were exposed to a daily dosage of 0.01 ppm of toxaphene for

TABLE 5. *Bioassays* of insect larvae* (Ischnura *spp. and* Enallagma *spp.) exposed to sublethal and lethal toxaphene dosages*

Trial no.	Toxaphene dosage (ppm)	Toxaphene percentage and formulation	Exposure period (hr)	Number bioassayed	No. of fish	TEST FISH: No. of fish surviving after (hr) 24	48	72	96	120	TEST FISH: Av. water temp. (°F)	CONTROL FISH: No. of fish surviving after (hr.) 96	120	CONTROL FISH: Av. water temp. (°F)
1	0.004	100% technical grade	120	50	4	4	4	4	4	4	70	4	4	70
2	0.01	100% technical grade	120	50	4	4	4	4	4	4	71	4	4	70

* *Lepomis cyanellus* employed as test fish.

a period of 384 hr. A 10-L sample from each culture was centrifuged, dried and ground. To each dried sample 20 ml of benzene were added and evaporated so as to extract any accumulated toxaphene and 10 ml of isopropyl alcohol were then added to the residues to dissolve any toxaphene which may have been extracted. The samples were reintroduced into 10 L of water and bioassayed. The results indicated that there was an insufficient quantity of toxaphene accumulated by the algae to incur a mortality among the test fish (Table 3).

Periphyton (composed of various algae, diatoms and ciliates) was exposed to a daily dosage of 0.01 ppm of toxaphene for 336 hr. The periphyton was then scraped from the inner surfaces of the aquaria and subjected to the toxaphene extraction procedures as mentioned above. Following addition of isopropyl alcohol to the periphyton residues they were reintroduced into 30 L of water and bioassayed for the presence of toxaphene. None of the test fish survived for longer than 24 hr (Table 4). In view of the results from the bioassay of planktonic algae it is suggested that periphyton may accumulate toxaphene by incorporating it into the mass rather than by absorption.

Damselfly nymphs were exposed to lethal and sublethal dosages of 100% technical grade toxaphene which was dissolved in isopropyl alcohol. These nymphs, 50 in each experiment, were fed to green sunfish. The results of the bioassays indicated that there was an insufficient quantity of toxaphene within the bodies of the nymphs to produce a mortality among the test fish (Table 5).

Results of the above experiments indicate that single, sublethal dosages of toxaphene are inadequate to produce accumulations in fish-food organisms which cause mortalities among the test fish. It is possible that dosages of this nature are detoxified before they can be accumulated or that they are detoxified below a threshold of accumulation. On the other hand chronic dosages may keep pace with the detoxification rate in laboratory acquaria and permit the accumulation of toxaphene by *Daphnia* and periphyton. It is probable that the latter dosages simulate the detoxification of waters which were heavily treated with toxaphene.

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The Effects of Rotenone and Toxaphene Upon Plankton of Two Colorado Reservoirs[1]

Limnologists and fishery biologists are constantly seeking methods of increasing fish production of inland waters. By one method, undesirable fish populations are eliminated by means of piscicides. The body of water can then be restocked with desirable fish.

The use of piscicides in any body of water may affect the plankton population. Such changes may in turn have marked effects on subsequent fish populations, since these organisms directly or indirectly constitute the food of fishes. This study is an attempt to analyze the effects of two piscicides, rotenone and toxaphene, upon plankton populations.

The investigation was conducted upon three reservoirs near Ft. Collins, Colorado. Reservoir Number Four, a single basin lake with a surface area of 60 acres and a depth of 15 ft, was treated with a water emulsifiable concentration of 60% toxaphene at a concentration of 0.1 ppm. Derris powder containing 5% rotenone was applied at a concentration of 1.0 ppm to Smith Lake, a single basin lake with a maximum depth of 11 ft and a surface area of 24 acres. A third reservoir, Reservoir Number Two, was retained as a control.

[1] A portion of a thesis submitted to the Graduate Faculty of Colorado State University, by the senior author in partial fulfillment of the requirements for the degree of Master of Science.

TABLE 1. *Range of chemical and physical observations in two experimental reservoirs and Smith Lake, from October 21, 1954, to September 30, 1955*

	Alkalinity Phenolphthalein	Alkalinity Methyl Orange	Oxygen % Sat.	pH
Chemical Data				
Res. 4	4–17	36–134	74–207	8.2–8.9
Res. 2	0–18	45–207	74–200	7.9–8.5
Smith Lk.	0–30	30–284	70–195	7.6–8.9

		Physical Data Water Temp. (°F)	Secchi Disc (in.)
Res. 4	Surf.	34.4–75.0	26.5–217.0
	Bot.	36.6–73.0	
Res. 2	Surf.	33.9–71.4	6.0–24.0
	Bot.	40.0–65.6	
Smith Lk.	Surf.	34.8–72.0	20.5–74.0
	Bot.	38.0–71.1	

Data for the study regarding the effects of rotenone and toxaphene were collected from October 21, 1954, to September 30, 1955. Plankton collections from the bottom and surface strata of the lakes were taken by means of a ten-liter Juday Plankton Trap. Numbers of plankton organisms per liter of lake water were determined by direct counts. All chemical and physical data were collected and compiled by standard limnological techniques. Plankton collections were taken biweekly and water samples for chemical analysis were collected monthly.

The turbidity of the two lakes decreased after treatment to the point that the bottom was visible, whereas the control lake remained turbid throughout the study period. Free carbon dioxide was detected in trace amounts in Smith Lake, on June 22, 1955, and in Reservoir Number Two on July 20, 1955. No other free carbon dioxide was detected in any of the lakes during the course of study. All other chemical characteristics are summarized in Table 1.

After poisoning, a luxuriant growth of *Potamogeton* occurred in the treated reservoirs. No doubt such a growth could have been influenced by the clearing of the water, and the added nutrients from the dead fish. No higher aquatic plants were observed in Reservoir Number Two, the control reservoir.

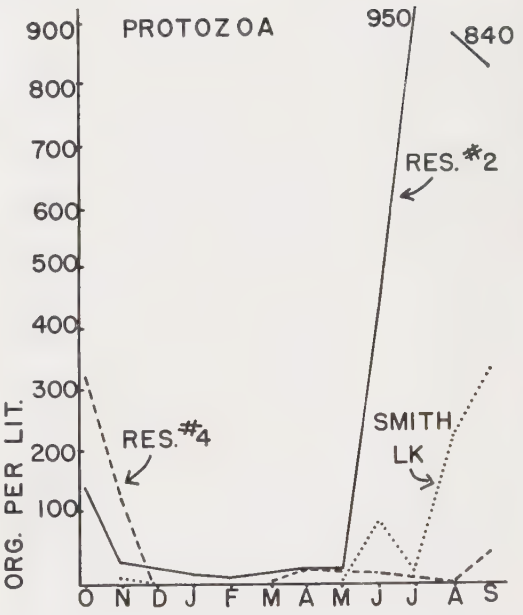

FIG. 1. Annual Cycle of Protozoa in Experimental Reservoirs from October 1954, to September 1955.

The plankton of the three study lakes were grouped into three categories, Entomostraca, Rotatoria, and Protozoa. The organisms listed below in each category are the same for each reservoir unless otherwise noted.

ENTOMOSTRACA
- *Cyclops*
- *Diaptomus*
- Nauplii of *Cyclops* & *Diaptomus*
- *Ceriodaphnia*
- *Bosmina*
- *Leptodora* (Res. 4 only)

ROTATORIA
- *Filinia*
- *Keratella*
- *Polyarthra*
- *Asplanchna*
- *Brachionus*

PROTOZOA
- *Ceratium*
- *Difflugia*

Following the application of toxaphene in Reservoir Number Four, protozoans decreased from high counts taken in October to zero in December. No protozoans were collected from the surface water until May. Similarly, numbers of protozoans dropped to zero in January after rotenone was applied in Smith Lake. The protozoans in Reservoir Number Two dropped to zero a month after the population declines of the other reservoirs. The protozoans in Reservoir Number Two increased, whereas those in the treated reservoirs remained at zero.

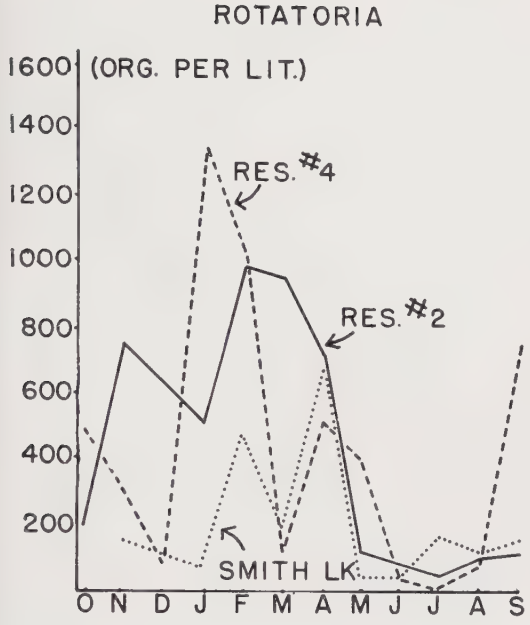

FIG. 2 Annual Cycle of Rotatoria in experimental Reservoirs from October 1954, to September 1955.

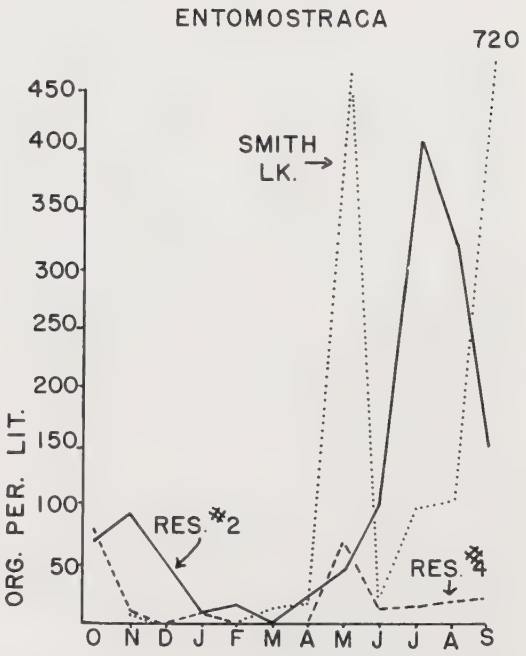

FIG. 3. Annual Cycle of Entomostraca in Experimental Reservoirs from October 1954, to September 1955.

The population pulse in the control reservoir during September was much larger than those in the other reservoirs (Fig. 1). It appeared that the fish toxicants may have accelerated the decline in numbers of protozoans in the treated lakes. Since the protozoans did not immediately repopulate the treated reservoirs, as in the case of the control reservoir, it would seem that the fish toxicants were inhibitory to protozoan growth. Hooper (1948) reported a decrease in the number of protozoans following treatment of Demming Lake, Minnesota. He attributed the apparent change in population to errors in sampling or normal fluctuations rather than effects of the poison.

Rotifer pulses were exhibited at the same time during the year in all three reservoirs. However, following population increases the rotifer population declined to lower numbers in the treated reservoirs than in the control reservoir. During the course of the study, Reservior Number Two, sustained a larger population of rotifers than Reservoir Number Four or Smith Lake. From these results, it is evident that the fish toxicants reduced the size of the rotifer population in the treated reservoirs. Likewise, Hooper's (1948) work indicated a reduction in the rotifer population after treatment of the Lake with rotenone. Comparing data of previous years, Brown and Ball (1943) found a considerable decrease in rotifers following poisoning with rotenone. *Keratella* survived treatment with rotenone in McCormick Lake, according to Smith (1940). In Potter's Lake, New Brunswick, where rotenone was used, Smith (1941) found *Keratella*, *Asplanchna*, and *Polyarthra* had survived (Fig. 2).

After the fish poisoning operation the populations of Entomostraca in the treated lakes decreased to zero, while the population in the control reservoir exhibited a pulse. No entomostracans were collected from the surface of Reservoir Number Four between December and April. Except for small counts of three organisms per liter in January and two per liter in February, no entomostracans were taken from the bottom stratum of Reservoir Number Four (Fig. 3). Following poisoning in November, no Entomostraca were collected from the bot-

tom stratum of Smith Lake until February. Reservoir Number Two, however, sustained populations of Entomostraca during that period. Furthermore, the decline in numbers was not as rapid in Reservoir Number Two as in Reservoir Number Four or Smith Lake (Fig. 4). The above conditions indicate that the Entomostraca were adversely affected by the fish toxicants. Various authors, Hamilton (1941), Hooper (1948), Brown and Ball (1943), Smith (1940), substantiate the findings that Entomostraca are killed by rotenone. Experiments by Hemphill (1954) and Moretti (1948) indicated that Entomostraca are also killed by toxaphene.

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YEARLY CHANGES IN THE PERU CURRENT[1]

The scientific investigation of the Peru Current in the past has been a sporadic effort, punctuated by the occasional visit of a British, American or other foreign research vessel, followed in a decade or two by a flurry of scientific reports. The systematic observation of changes in the current system, so essential to an eventual understanding of its normal and abnormal functioning, has been almost nonexistent. Fortunately, nearly 20 years ago, Dr. Erwin Schweigger of the Peruvian Compañia Administradora del Guano had the foresight to initiate the collection and compilation of sea-surface temperature and weather observations from Peruvian vessels, so that there exists, sparse though it may be, a record of ocean changes since the year 1939.

The accumulated data have been published (in the recently discontinued *Mapas Mensuales* of the Guano Administration) by half-degree squares, in most of which too few data are available to permit the calculation of meaningful averages. However, along the coastal shipping routes it is possible to select for each parallel of latitude a one-degree square containing the maximum number of observations (Fig. 1). I have calculated mean surface temperatures for these squares for each month of each year for the period 1939 through 1956. As shown by the inset, the annual range of temperature varies from about 4°C at 12°S to nearly 6°C at 4°S.

The grand averages of these data are shown in an isogram (Fig. 2) which illustrates the seasonal change of surface temperature along the Peruvian coast. Highest temperatures, up to more than 22°C, are found farthest north (*i.e.*, nearest to the

[1] Contribution from the Scripps Institution of Oceanography. Paper delivered at meeting of Pacific Section, American Society of Limnology and Oceanography, San Diego, 17 June 1959. The work was supported in part by the Peruvian Consejo de Investigaciones Hidrobiológicas, and by the Office of Naval Research and the Bureau of Commercial Fisheries through contracts with the University of California.

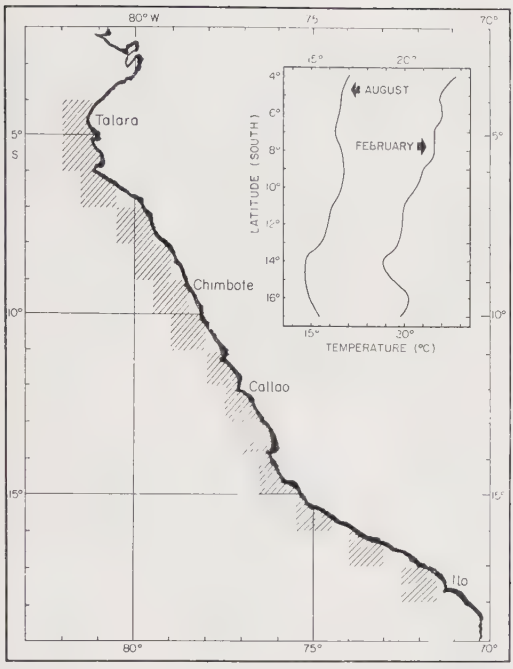

FIG. 1. One-degree squares used in analysis. Inset shows average February and August surface temperatures for period 1939–1956.

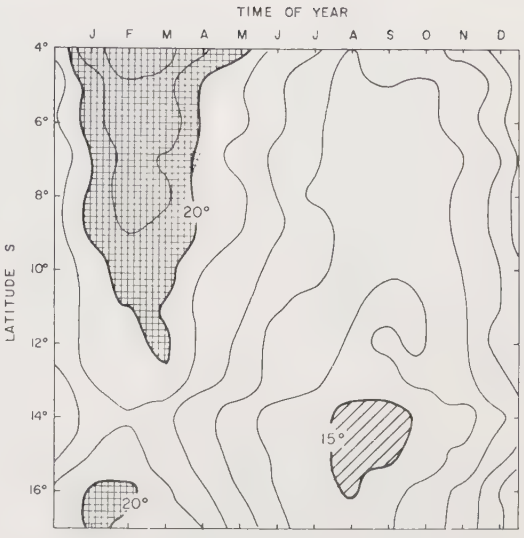

FIG. 2. Average surface temperature for one-degree squares for period 1939–1956, from Wooster (1960).

equator) in the summer, February and March being the months in which warm waters extend farthest south. The summer warming in southernmost Peru is separated from that in the north by colder water at 14° and 15°S. Indeed, during all months of the year lowest temperatures are found at those latitudes. Only there do average temperatures fall below 15°C during the winter cool period, which seems to be best developed in August, September and October. The most conspicuous event of the year appears to be the oncoming of summer, with temperature changes of several degrees per month, whereas during the rest of the year the average rate of change is one degree per month or less.

The detailed yearly isograms (Figs. 3 and 4), as one would expect, are much more irregular in appearance, primarily because of the much smaller number of observations involved (when less than 6 observations were available for a given square, the average was not used). The alternation of seasons is still plainly visible. Obviously not all years are the same, and the differences between years are often striking.

For example, waters warmer than 20°C were first observed off northern Peru as early as November, 1940, two months before usual, and by January, 1941, the warm region extended southward to at least 16°S, with temperatures from 2°C to nearly 5°C higher than normal. In February and March temperatures in excess of 25°C were observed north of 9°S, and the presence of water several degrees warmer than usual persisted through June. Disappearance of the warm regime was abrupt, with surface temperatures dropping 2–3°C in a month to the north of 12°S.

This "El Niño" of 1941 has been described by several investigators (Schweigger 1942; Sears 1954; Lobell 1942; Vogt 1942). Its principal characteristic, as shown by these temperature charts, is the marked increase in southward penetration, duration and intensity of the annual summer warming off the Peruvian coast. Another year, 1953 (Fig. 4), which has been considered a Niño year (Posner 1957), shows some of the same features, although to a smaller extent.

Other years are clearly colder than usual, the period 1950–51 presenting an interest-

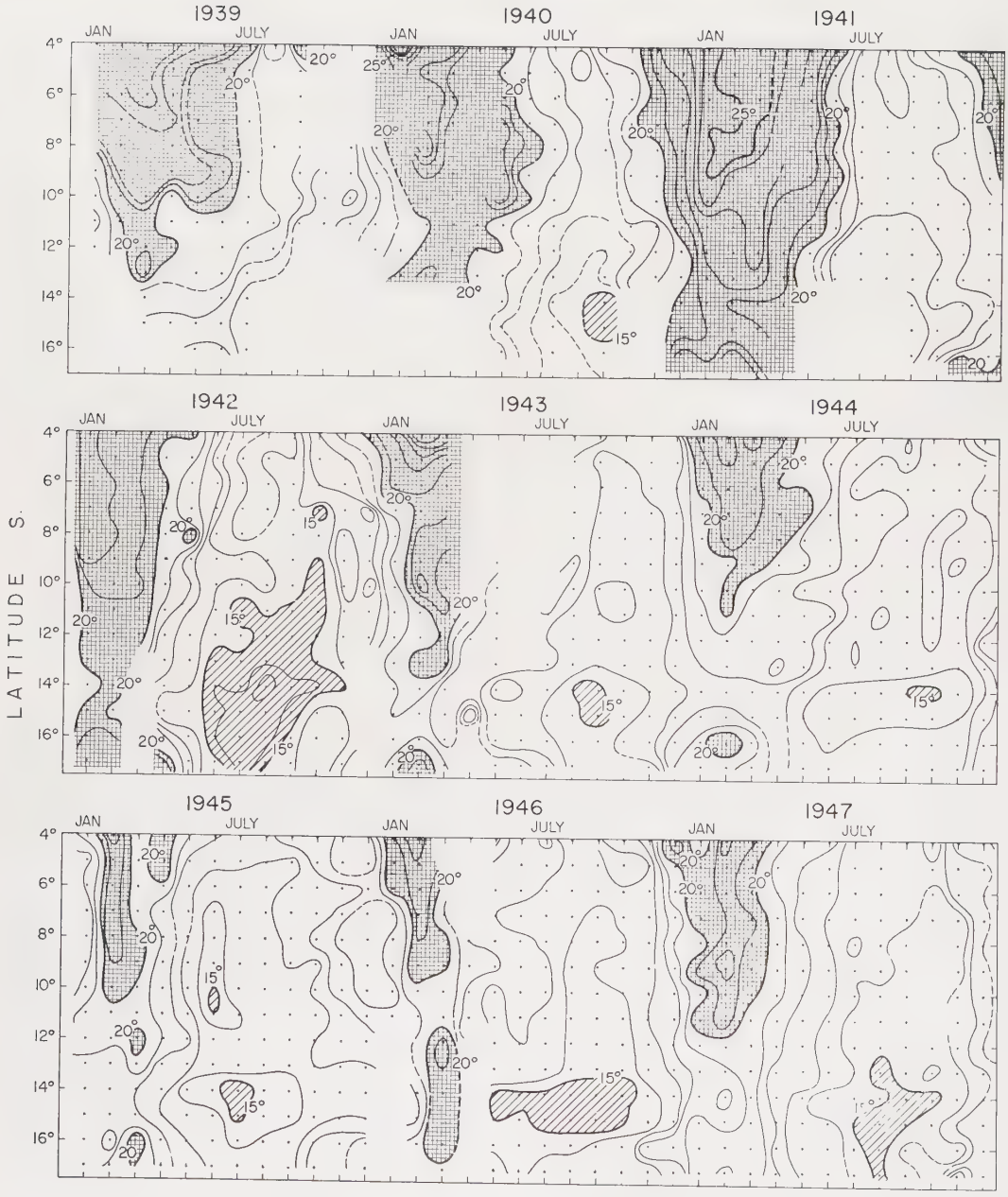

FIG. 3. Average surface temperature for one-degree squares, for individual years 1939 to 1947.

ing example. The summer warming of 1950 was notably reduced; even in northern Peru, surface temperatures remained below 20°C, from 2° to 3.5°C cooler than usual. The winter cold regime was well developed, and the warming of early 1951 was again weak. Then conditions changed rapidly in May, 1951, with a wintertime development of waters warmer than 20°C extending as far south as 13°S, a feature apparent nowhere else in the available record.

Although it is obviously impossible to account for all the features of these records,

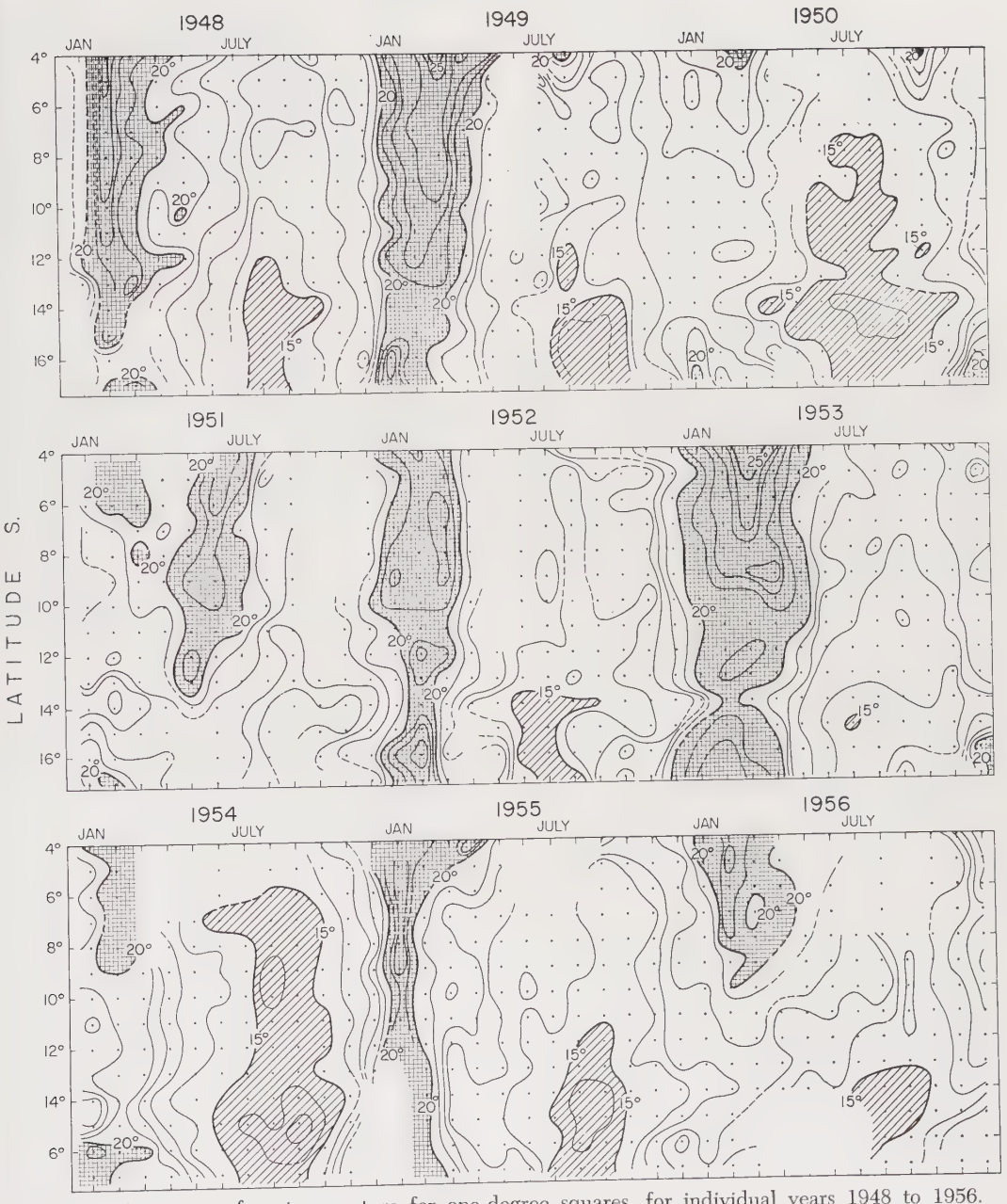

FIG. 4. Average surface temperature for one-degree squares, for individual years 1948 to 1956.

the general trends are not so inexplicable. During the winter the strongly developed southeast trades are responsible for the optimal development of upwelling, so that low temperatures prevail along the entire coast. The zone of lowest temperatures, at 14° to 15°S, is located where there occurs a 30° change in direction of the coast line relative to that of the average wind. During the summer the intertropical convergence is at its southernmost latitude, as is the north boundary of the Peru Current, and the weakened southeast trades are less effective in producing upwelling.

If this picture of seasonal change in the Peru Current region is essentially correct,

it seems reasonable to expect that the major non-seasonal changes are linked to major variations in the surface wind circulation. The events of 1941 could be interpreted as indicating an abnormal weakening of the trade wind circulation (Wooster 1960). The low temperatures of 1950–51 could result from stronger than usual southerly winds along the Peruvian coast. The recent work of Bjerknes (1959), Roden and Groves (1960), Roden and Reid (in press) and others tends to support such an interpretation, although temperature-wind correlations do not yet give as clear-cut an answer as one might wish. Unfortunately in the southern hemisphere the supply of reliable historical weather data seems to be small. If usable data can be located, it seems likely that the picture of yearly change suggested by these surface temperature isograms will be relatively simple to explain.

FOOTNOTE

Since completion of this work, Schweigger (1959; Fig. 6 A, 6 B) has published surface temperature isograms for the period 1948–1956 based on a somewhat different interpretation of the same data.

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Light Attenuators for Use in Phytoplankton Photosynthesis Studies

A study of the response of phytoplankton to variations of light intensity is becoming increasingly important in primary productivity research, both in lakes and in the ocean. In all such work we need to illuminate bottles of water containing algae to a light intensity which is a known fraction of that of an incident light source which may be daylight or some artificial form of illumination.

It is more difficult than might be supposed to contrive a convenient attenuator, the coefficient of which is easily reproducible, can be varied over a wide range and is constant with time. The attenuator should be robust, unaffected by water and truly neutral in its spectral behavior. Fogged photographic film or plates, even with protective coatings, have few of the above desirable characteristics.

If light is blocked off by wire gauze of fine mesh size the attenuation is truly neutral and the system can be made robust and immersed in water for reasonable periods of time with no deterioration. Furthermore, provided that the mesh size of the gauze is sufficiently small, the effect of more than one layer of gauze is additive, that is to say if the fractional transmission of one layer of gauze is T the attenuation of two layers is approximately T^2 *etc.*, up to at least four layers.

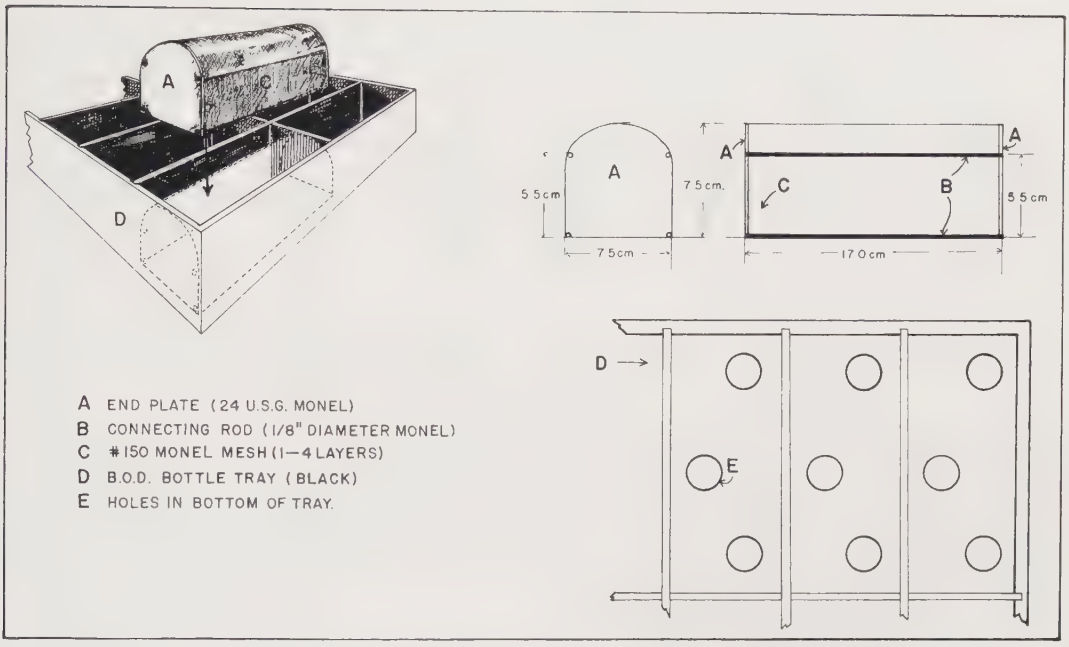

FIG. 1. A light attenuator, showing also its use in a tray from a water-cooled incubator.

This forms the basis of the neutral filters shown in Figure 1. The "light incubators" used by us for photosynthetic work are designed to hold black-painted trays with perforations in their base which allow cooling water to flow up through the equipment. The trays, which are illuminated from above, hold 300-ml B.O.D. (biological oxygen demand) bottles which we use for both oxygen and C^{14} photosynthetic rate measurements.

The filters consist of one or more layers of 150-mesh Monel metal screen wrapped over a Monel metal frame of rods and plates, as shown in the diagram, and soldered to the bottom rods and to the endplates. B.O.D. bottles fit snugly into this assembly which will rest in the compartments of incubator trays (as shown) or can be used anywhere where a flow of cooling water is available. Bubbles may tend to collect on the outer surface of the mesh when water is flowing but can be easily dislodged. As a precaution it is perhaps best to pass cooling water through a bag made of 150-mesh Monel screen before it enters the incubator but we have found no evidence of clogging or deterioration of filters in continual use throughout one summer. They should be rinsed with fresh water and stored dry.

The percentage transmission of light is surprisingly uniform over the base of a filter and amounts to about 35% for one thickness of screen, 13% for two thicknesses, 5% for three thicknesses and 2% for four thicknesses. The variation from filter to filter does not exceed 5–10% of the absolute amount and changes very little with time. We do not recommend the use of five layers of screen but a flat "filter" of 150-mesh Monel may be placed over one or more compartments in a tray. This effectively multiplies the transmission of each filter thus covered by a further 0.35.

The gap between 100% transmission and the first Monel filter (with 35% transmission) is rather large. However, a filter with about 65% transmission can be constructed using galvanized screen made from 16 × 12 mesh S.W.G. 33 wire. Doubtless similar materials could be found to give transmissions greater than 65%. Gal-

vanized iron wire, protected by an acrylic spray treatment, has proved to have adequate corrosion resistance.

Attenuators should be calibrated *in situ* whenever possible. The exact fraction of the light cut out by the screens will vary somewhat with the equipment used and the nature of the light source, in particular whether or not the light reaching the screens is parallel or diffuse.

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The Ecology of *Urnatella gracilis* Leidy: Phylum Endoprocta

distribution

Rogick and Van Der Schalie (1950) erroneously referred to *Urnatella gracilis* as being exclusively North American. Damas (1938), however, reported its occurrence in the Meuse River in Belgium, and this is apparently the only record of its occurrence outside of North America. *U. indica*, the other species within the genus, was described by Seshaiya (1947) from specimens collected in southern India. These two species constitute the reported freshwater endoproctan fauna of the world.

Leidy (1851) described *U. gracilis* from specimens taken in the Schuylkill River in Pennsylvania. Subsequent collections at the original collecting site were taken by several authors including Allman (1856). Leidy (1884) mentions its occurrence in the Scioto River, Ohio, on the basis of remnants of specimens found on a unionid shell sent to him by Lea in 1883.

At the turn of the century, Kofoid (1898, 1908) recorded *U. gracilis* from the Illinois River near Havana, Illinois. In several papers between 1921 and 1928, Richardson also recorded the species from the Illinois River. Specimens have been reported from the Licking River in Kentucky by Williams (1930). Rogick (1936) reported the species from several Lake Erie locations, and in the same paper she commented on specimens collected in the Clinton River in Michigan. Rogick and Van Der Schalie (1950) reported *U. gracilis* from the Grand River above Ionia, Michigan, and from the Tippecanoe River near Pulaski, Indiana. Davenport (1904) mentioned the occurrence of specimens in the Mississippi Valley but gave no evidence of collections or authority.

There are two observational reports of *U. gracilis*. One of these was by Dr. B. B. Harris at Lake Dallas, Texas, and the other by Dr. R. C. Osburn from specimens seen in the Mississippi River near Fairport, Iowa.

My first collections of *U. gracilis* were taken at Mile 598 (598 miles downriver from Pittsburgh, Pa.) in the Ohio River just above Louisville, Kentucky in September, 1958. Examination of bottom collections taken the previous year disclosed many specimens of *U. gracilis* at Mile 596. Since then I have taken additional specimens from bottom samples at Miles 113.7, 326.0, 443.8, 458.0, 600.0, 617.0, 618.0 and 630.0. Thus, it is to be found over a 500-mile stretch of the Ohio River.

All of my collections were made in regions of unidirectional currents flowing between ½ and 5 m.p.h. All other collections on record, with the exception of the Lake Erie collections of Rogick and Van Der Schalie (1950), were taken from flowing waters.

U. gracilis is characteristically found attached to rocks, sticks, and molluscan shells. I have found as many as 1,000 or more specimens on a 3-in. rock. Many specimens were taken from *Amblema* shells and a few were found on the shells of *Pleurocera caniculatum*. All specimens were found in regions of coarse gravel, rubble or boulders. None were found on sand or mud bottoms.

Hydra americana was found associated with *U. gracilis* on practically all occasions.

TABLE 1. *Some chemical and physical factors of the Ohio River between Miles 113.7 and 617.0*

Date	Mile	pH	O_2 (mg/L)	O_2 (%)	Free CO_2 (mg/L)	M.O. alk. (mg/L)	Temp. (°C)
1 X 57	596	7.5	6.00	67.9	4.8	80.0	22.2
31 X 57	596	7.6	8.70	82.9	3.5	74.0	13.7
4 IX 58	598	7.4	12.22	160.0	7.2	94.0	30.0
20 IX 58	598	7.8	5.90	78.0	2.3	78.0	22.5
24 X 58	617	7.3	4.20	44.6	7.7	81.0	18.6
11 VII 59	475	7.7	6.20	104.0	2.5	67.0	29.0
14 VII 59	303	7.1	6.50	83.4	6.7	44.0	29.0
14 VII 59	310	7.5	7.50	94.4	2.7	45.0	28.4
14 VII 59	318	7.8	8.40	110.1	1.3	44.0	28.5
14 VII 59	326	7.7	7.70	96.9	1.6	43.0	28.4
28 VII 59	113	7.7	9.70	124.0	.88	23.0	30.0

Other associates were *Paludicella articulata*, *Canthocamptus* sp., *Spongilla* sp., *Vorticella* sp., *Carchesium* sp., *Stentor* sp., one colony of *Cordylophora lacustris* and a flosculariid rotifer.

The potamoplankton occurring in the regions in which *U. gracilis* was found consisted of more than 50 species of typical river diatoms, blue-green and green algae, protozoans, rotifers, cladocerans and copepods.

Examination of several specimens disclosed that *U. gracilis* was feeding on the diatom, *Melosira varians*, and two species of green-algae, *Pediastrum duplex* and probably *P. simplex*. Richardson (1921) reported this endoproctan feeding on diatoms in the Illinois River.

It can be seen from Table 1 that *U. gracilis* can live in regions subject to wide variations in physical and chemical conditions. Many of the specimens taken at Miles 617 and 630 in the Ohio River were found in polluted regions containing as little as 4.2 mg/L of dissolved oxygen during the day and undoubtedly much lower values at night. Saturation of oxygen in this region reached a daytime low of 44.6%.

The pH values ranged from 7.1 to 7.8 but 7-year data compiled by the Ohio River Valley Water Sanitation Commission indicate a range between 4.8 and 8.9.

On collection dates, the temperature of the water ranged from 13.7 to 30°C; however, temperatures as low as 1.0°C have been recorded over the entire length of the collecting area.

The upper portion of the Ohio River contains little $CaCO_3$, as indicated by the low total alkalinity of 8 mg/L from the same 7-year analyses of the Sanitation Commission. The lower region is much more alkaline, and values of 142 mg/L have been recorded. At the sites where *U. gracilis* was taken, the alkalinity ranged from 23 to 94 mg/L.

Turbidity varied from 2 to 2400 mg/L at the collection sites. The latter value would seem to be destructive to most small filter-feeders. However, the regions in which this value was obtained supported large numbers of *U. gracilis*.

GENERAL CONSIDERATIONS

Davenport (1904) stated that the Illinois River specimens branched more profusely than the original Pennsylvania specimens. I have found a great deal of branching in the Ohio River forms; one colony had 5 main branches and many side branches which produced a total of 24 calyces. Also, the Ohio River specimens which I examined had a maximum of 8 segments whereas those examined by Leidy had a maximum of 18 segments. If specimens are still available from the Philadelphia site, the two populations should be compared since there may be an ecophenotypical difference involved.

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AN ENLARGED CLARKE–BUMPUS PLANKTON SAMPLER[1]

The plankton sampler described by Clarke and Bumpus (1950), and improved by Paquette and Frolander (1957), is of limited use in oceanic areas of low plankton abundance. In such regions, relatively large volumes of water must be strained to increase the probability of catching a representative sample. To overcome this difficulty a similar instrument of approximately twice the diameter of the original model was designed. This quadruples the amount of water strained per unit time but changes as little as possible the well-proven operating principles of the earlier instruments.

The new design essentially involves only a change in scale, although modifications from the original form were required to avoid the use of castings, and to maintain ruggedness and stiffness without the accumulation of excess weight. The resulting instrument is illustrated in Figure 1.

The tubular body is made from a 5-in. length of seamless brass tubing 10 in. in inside diameter, with a ⅛-in. wall thickness. As in the earlier instrument, the body is supported within the frame at its forward end by a pair of axles, with the axis of rotation passing through the center of a half-circular ring on the gate. As before, this half-ring engages an L-shaped release lug on the index shaft, which in turn controls the two motions of opening and closing of the gate. The planing vanes, which give the body a horizontal aspect during operation, are of 1/16-in. stainless steel sheet, 8 in. long by 5 in. wide and extend 5 in. aft of the body tube. They are given a tilt of 15° with respect to the longitudinal axis, and a dihedral angle of 10°.

The frame is fabricated from ¼- × 2-in. brass flat bar held together with ¼-20 stainless steel socket-head cap screws. Reinforcement is provided at corners and other points by silver-soldered ⅜- × ½-in. brass blocks tapped for cap screws. The junctions of the upper and lower angular

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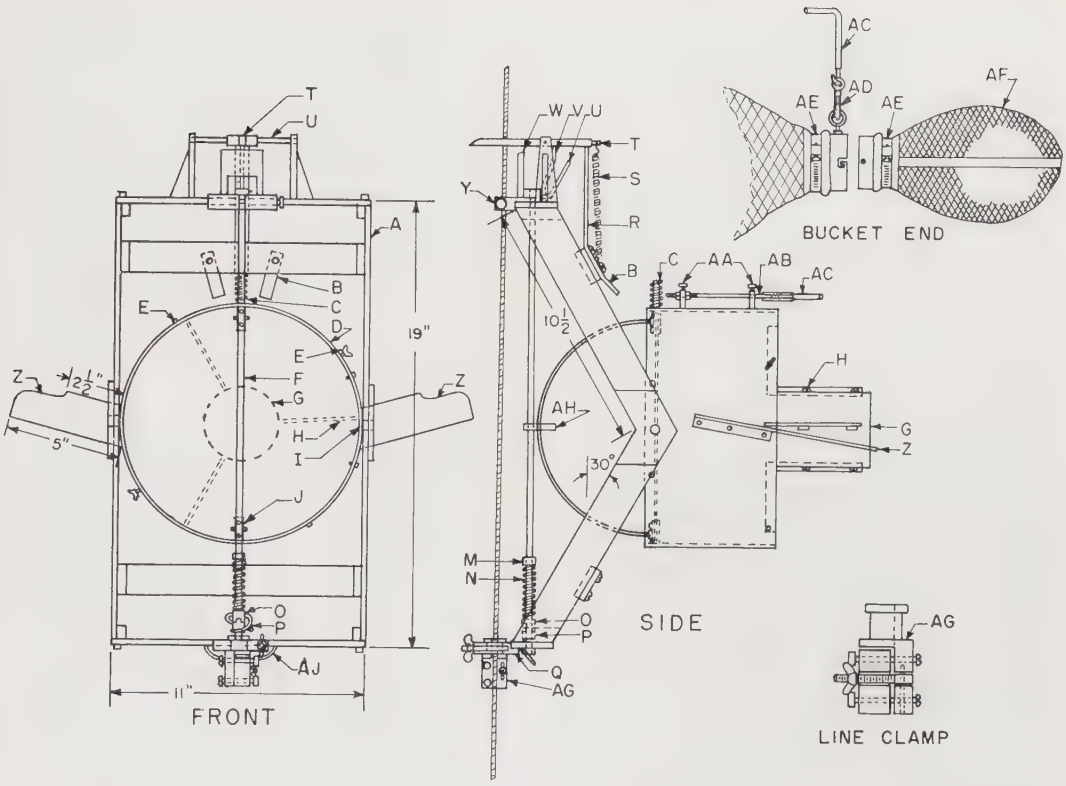

FIG. 1. Enlarged Clarke–Bumpus plankton sampler and improved line clamp. See Parts List below.

PARTS LIST

A Frame; B Stop; C Gate Spring; D Body; E Bayonet pin (2 long, with wing nut; 2 short); F Half-ring; G Flow Meter; H Flow Meter support (3); I Main axle; J Gate axle (2); K Gate release lug; L Index shaft; M Dog-clutch thrust collar; N Dog-clutch thrust spring; O Dog-clutch, upper member and helical messenger-release fingers; P Dog-clutch lower member; Q Line-clamp bushing; R Release-fork stop; S Release-fork spring; T Release-fork; U Index assembly, center pin; V Index assembly actuating pin; W Anvil; X Index assembly, detent pin collar; Y Upper line-attachment assembly; Z Vane (2); AA Boom support posts; AB Boom socket; AC Boom; AD Harness snap; AE Hose clamps; AF Sample sack; AG Line clamp; AH Release lug; AJ Guard

sections of the frame are strengthened by silver-soldered V-shaped pieces.

The release mechanism (including the fork-support, fork mechanism, fork restraining spring, index mechanism, dog-clutch, helical messenger-release fingers, and the corresponding slots in the bottom plate) is identical with that described by Paquette and Frolander (1957). Changes in construction lie only in the use of ¼-in. stainless steel rod for the index shaft and in the use of an enlarged release lug.

The gate is made of ⅛-in. sheet brass, the axle of ¼-in. brass rod, and the gate spring of 24 turns of 0.056-in. inconel wound on a 5/16-in. mandrel. The half-ring is made of 3/16-in. phosphor bronze spring wire. The guard, AJ, which prevents messenger lanyards from hanging up on the line clamp, is made from 3/16-in. rod.

An innovation is the installation of a Tsurumi T. S. plankton-net flow meter[2] instead of the original built-in propeller. The meter is adapted to the after end of the body by means of three L-shaped brass

[2] Tsurumi Seiki Kosakusho Company, Ltd. 1506 Tsurumi-cho, Tsurumi-ku, Yokohama, Japan.

arms, which are slotted into the after edge of the cylinder and silver-soldered. These arms bear holes to match the supporting eyes on the flow meter. The meter is fastened to the arms by means of machine screws. The Tsurumi meter has several advantages over the propeller–odometer combination. Besides being inexpensive, it has a completely flooded gear-driven dial register and so avoids the friction and corrosion problems associated with odometers. It has automatic stops which are an advantage in preventing propeller movement in the wind or during reverse motions.

The nets are 42 in. long and have been manufactured by the Puget Sound Workshop.[3] They are fastened to light, grooved, metal rings by means of stainless-steel hose clamps that have been lengthened by cutting and inserting an additional strip of metal. The rings fasten to the after end of the main body by means of four bayonet catches, two of which are secured by captured ¼-20 wing nuts.

As in the original instrument, the cod-end is supported by a metal boom, which prevents the net from wrapping around the supporting wire. This boom has been made of ¼-in. stainless steel rod, jointed just aft of the main body and about half way along its length. The coupling sleeves are made in such a way that ⅜-in. (or more) of unthreaded rod is supported inside the coupling to prevent breakage by concentration of stress at the thread. A ¼-in. rod has been found a little limber, and it would be well to consider making at least the butt section of somewhat larger rod or tubing. The end of the boom carries a harness snap, which connects into a wire loop on the bayonet coupling of the cod-end.

The cod-end has been modified from the conventional steel bucket. It consists of a small mesh sack joined to the trailing end of the net by means of a pair of grooved bayonet rings. The body of the net and the cod-end are fastened to the female and male partners of the pair of rings by means of lengthened hose clamps applied over the grooves. This design has these advantages: the sack is easier to attach and remove, it is less subject to damage than the metal bucket, and it is cheaper.

The sampler is fastened to the wire in the conventional way, using a spring-loaded retainer pin at the top of the instrument, and a cylindrical clamp, about which the sampler rotates, at the bottom. The retainer pin has a short, threaded portion to lock it in place.

The line clamp at the bottom of the instrument also has been improved and is illustrated in Figure 1. The principal difficulty with the old design was that it would fit only one size of wire rope. Further, the cylindrical portion serving as a bearing for the rotation of the sampler, expanded with an increase of wire size, and a slight change in actual wire diameter occasionally resulted in jamming. The new clamp has a cylindrical section independent of the clamping section. The wire passes through a ¼-in. round-bottomed slot in the cylindrical section and then is clamped between two flat surfaces, whose separation may be adapted to various wire sizes by adjustment of the two knurled screws. Difficult to see in the drawing is the fact that the upper knurled screw engages a slot rather than a hole in the movable plate. Release of the clamp involves loosening the wing nut and swinging away the clamping screw, after which the movable plate may be pivoted on the lower screw to release the clamp from the wire.

The sampler has been tested over a period of about a year, during which over 150 samples have been taken. In only 6 cases did the gate mechanism fail to operate. For the most part, these failures were attributed to the messengers not sliding down the wire; this in turn was due to the presence of burrs or jellies on the wire. The sampler can be tripped at wire angles from 0 to 75°. When towed at speeds of two to three knots for about 30 min. the sampler strains approximately 80–100 m^3 of water. These operating conditions have been found to yield samples that are reasonably representative, yet in most in-

[3] 3311-110th Ave. S. E., Bellevue, Washington.

stances small enough to be counted without entailing extensive periods of time.

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A BOTTOM SEDIMENT SAMPLER

As we had not been entirely satisfied with commercially available dredges, we were led to develop the dredge shown in Figure 1. A feature of some novelty is the trigger mechanism which is actuated when the dredge hits the bottom and the line slackens. The weight A, until then held up against a stop on its supporting shaft B, is free to drop, under its own weight, and to push aside the dogs that have held the jaws open. As soon as they are free of the hardened steel steps in B, the tension springs cause the jaws to close with considerable force. The use of this trigger

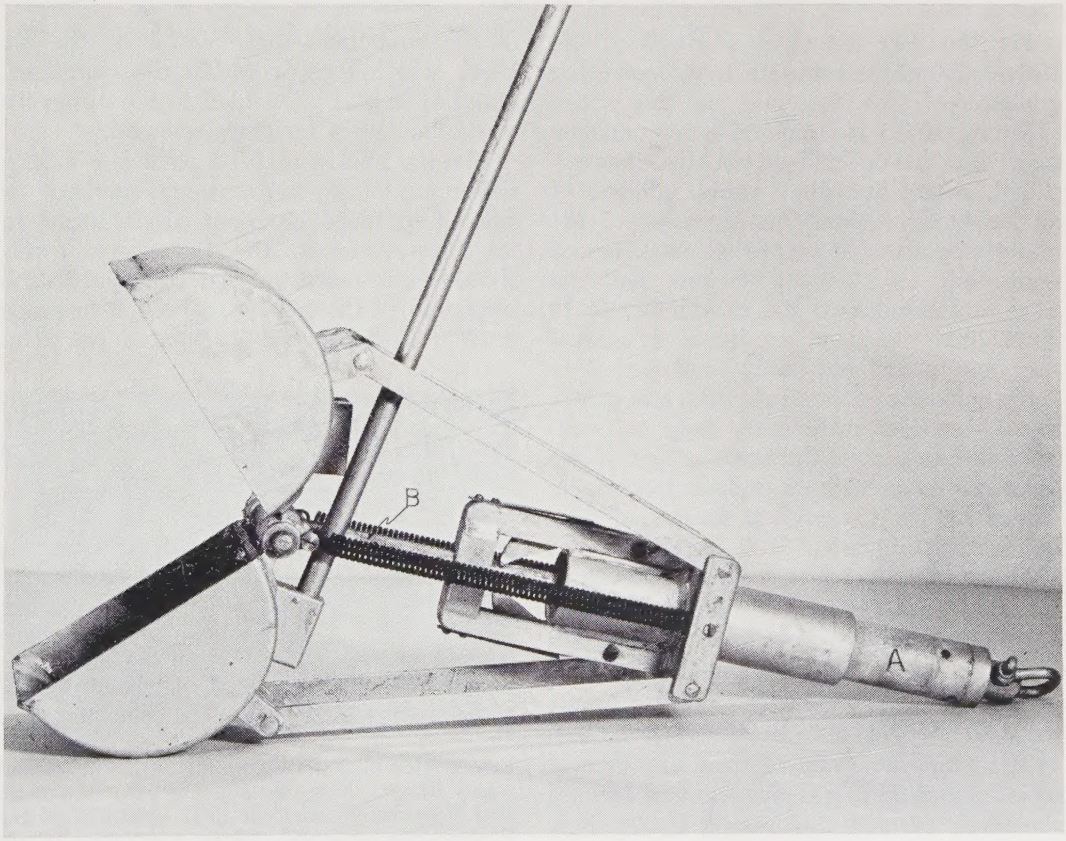

FIG. 1. Dredge in cocked position with one cocking lever in place.

mechanism permits a neat instrument without outboard appendages (*e.g.*, a trigger foot), and the triggering is very reliable. The dredge does not need to fall freely through the last few feet; indeed it is better to lower it at normal speed so that it is upright when it strikes the bottom. Crossed levers inserted in opposing positions in each cup allow safe and easy cocking when the instrument is suspended preparatory to lowering the wire. One's hands need be near the jaws only for removing the sample and at this time a stop prevents accidental tripping. The lips of the jaws are made of hardened steel welded into place and ground to give a tight, overlapping joint when the jaws close.

Used in bottom types ranging from gravels, sands, gelatinous clays and soft ooze, the sampler has proven reliable in depths up to 700 ft, and consistently takes samples of 2500 cc or more. In gelatinous materials, the samples are cut out cleanly enough that relatively undisturbed sections of the first 5 in. can be secured. However the sampler, in contrast to the Ekman dredge for example, tends to disturb the surface inch of soft oozes, and in these circumstances is not so suitable either for collection of bottom organisms. At the other extreme, coarse cobbles up to $4 \times 4 \times 3$ in. have been taken, and good samples of coarse sand secured as well.

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A Device for Successive Ice Penetration

For the past ten years students at the University of Massachusetts have been using a simple device designed by the writer (Nutting 1948) for successive ice penetration. The "ice bucket" as it is affectionately called, makes possible: rapid removal of an ice block without derangement of the underlying water mass, precise marking and resampling of identical stations, and the easy replacement of the sampling site to its original ecological condition. It is, further, low in cost and easily constructed.

Ice buckets may be made from any sturdy metal container sufficiently deep to penetrate the maximum thickness of ice of the pond or stream to be sampled. Containers should be wide enough to insure an adequate sampling area. It is important that the outside of the container be smooth since this is the surface which should slide free on removal. In our experience garbage pail liners or enamelled metal waste baskets, such as the one shown in Figure 1, make the best ice buckets. Remove the bottom of the container, peening flush any tabs of metal or rolled edges that project beyond the outer surface. Spot weld 1″–3″ angle irons at various levels on the inner surface of the container—these will hold the ice block which freezes within the container. Handles should be welded to the upper lip of the bucket to facilitate removal.

The ice bucket may be placed in a hole in the ice cut by conventional methods or floated in place on foam plastic prior to ice formation. In the first case dowels should be inserted in holes cut beneath the upper lip of the bucket to hold it in place until it is frozen in. If floated on foam

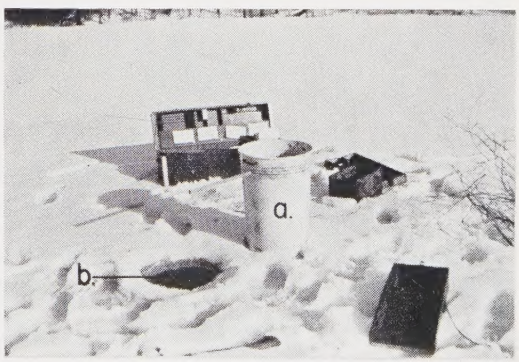

Fig. 1. Ice bucket (a) made from an enamelled waste basket. Note clear penetration (b) with area immediately adjacent little disturbed in the process of ice removal. (From MacKenzie, C. L. 1955, MS Thesis, University of Massachusetts.)

plastic an anchor must be provided for the bucket to insure precise location of the sample station. The plastic may be removed after freeze-in.

Two methods of removal have been successful: 1) The outer surface of the bucket may be greased with a good grade of vaseline prior to freeze-in. A sharp rap on the top of the bucket is usually sufficient to free it from the surrounding ice. 2) A propane torch can be used to heat the metal of the bucket thus releasing it from the ice. In either case, before removal, a metal rod must be heated or hammered through the ice block within the bucket to the level of the water so that a release vent is provided for water trapped within.

Two "cautions" in the use of the ice bucket should be mentioned here. First, the rim of the ice bucket should extend 3 to 6 in. above the ice to prevent freeze-over in case of melting snow or winter rains. Second, a float should be attached to the rim of the bucket so that it may be retrieved in case of "January thaws" or spring melt.

The ice bucket has been found in practice to 1) obviate the transportation of heavy, complicated gear for ice penetration; 2) decrease the time required to penetrate the ice with conventional methods by over one-half; 3) reduce turbulence of breakthrough to such a degree that accurate titrations of oxygen may be readily obtained (Nutting 1948); 4) speed up the entire sampling process so that closure may be effected without undue disturbance to the ecology of the pond; 5) make possible the rapid return of the sample station to its original ecological condition.

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